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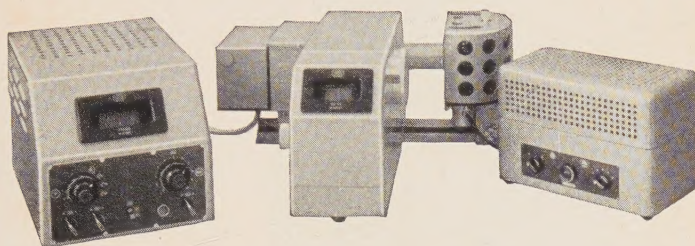
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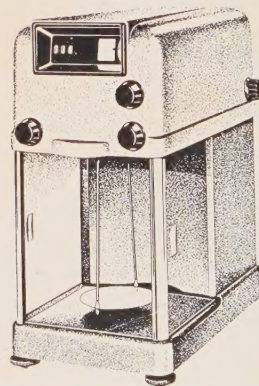
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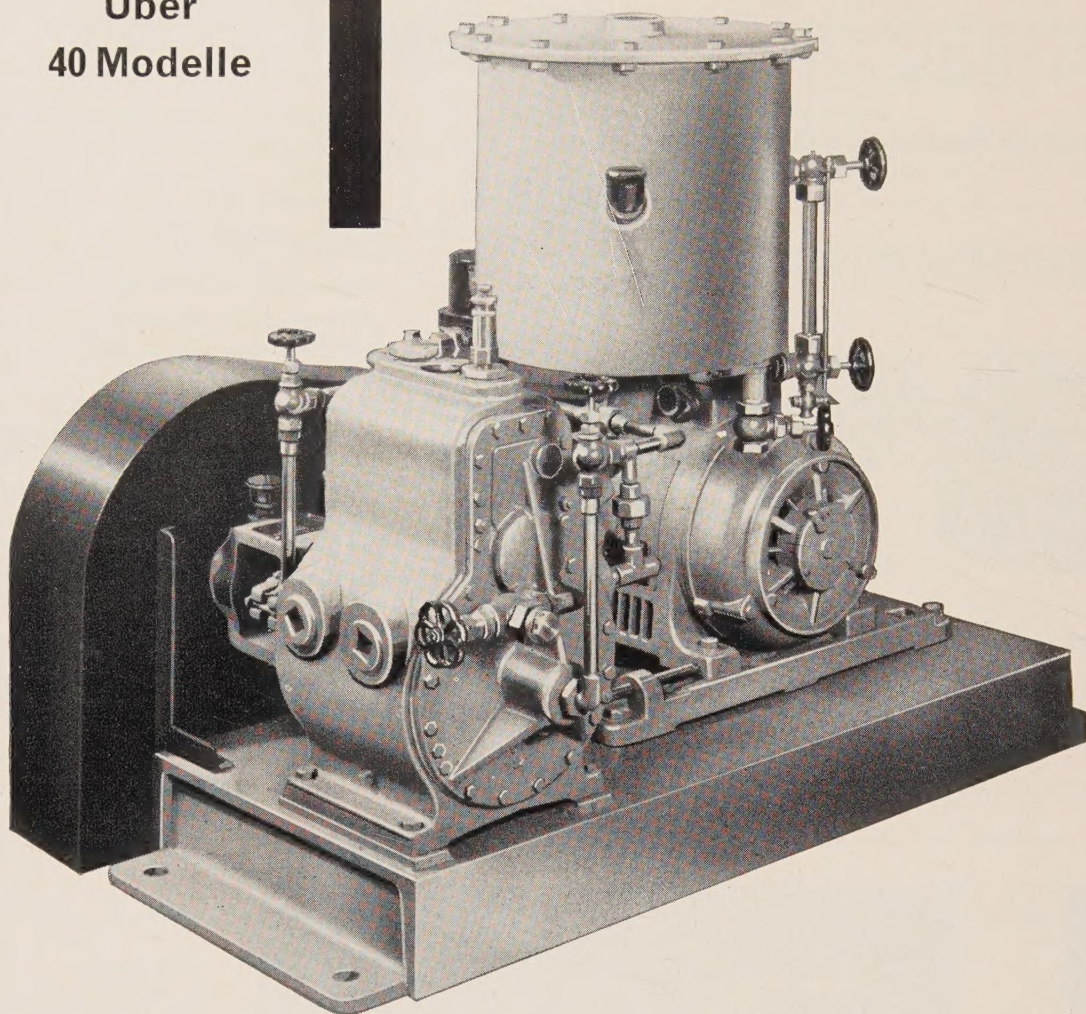
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Les bases cytologiques de l'hérédité «relativement» liée au sexe chez les mammifères

Par ROBERT MATTHEY, Lausanne*

On sait que, dans la grande majorité des cas, les mâles des mammifères sont digamétiques ($X-Y$) et les femelles monogamétiques ($X-X$). L'opinion classique veut que les gènes portés par chacun des hétérochromosomes X et Y du mâle soient rigoureusement liés au sexe: les filles seront seules à recevoir l' X paternel et tous les gènes qu'il porte; les fils hériteront du chromosome Y et de la totalité de son stock génique (fig. 1).

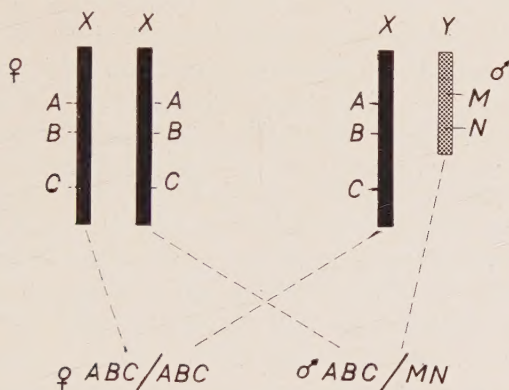


Fig. 1. L'hérédité strictement liée au sexe: les gènes A, B, C sont transmis par les chromosomes X , les gènes M, N par l' Y .

Au point de vue cytologique, les observations faites de 1920 à 1934 par divers chercheurs autorisaient les conclusions suivantes: 1° au cours de la période d'accroissement des spermatocytes, l' X et l' Y manifestent une hétérochromatie positive très marquée et évoluent au sein d'une vésicule hétérocaryosomique compacte; cette évolution, malaisée à saisir dans le détail, est radicalement différente de celle des bivalents autosomiques et ne s'accompagne pas d'échanges entre partenaires. 2° A la métaphase I, l' X et l' Y ne sont pas reliés par un ou des chiasmata, mais généralement par un connectif étiré représentant un reste de la substance vacuolaire de l'hétérocaryosome. 3° A l'anaphase I, l' X et l' Y se séparent (préréduction); les cytes II reçoivent donc, soit l' X , soit l' Y , qui, à l'anaphase II, se divisent longitudinalement selon le mode mitotique. 4° La préréduction étant le cas général, une exception est connue:

chez les *Apodemus*, à la suite d'un travail d'OGUMA¹ (1934), il a été montré que la postréduction est constante: à l'anaphase I, l' X et l' Y subissent une division longitudinale: les cytes II reçoivent chacun une chromatide de l' X et une de l' Y . La séparation des deux hétérochromosomes n'est donc effective qu'à l'anaphase II.

DARLINGTON, HALDANE et KOLLER² (1934) ont, les premiers, attiré l'attention sur la possibilité d'une hérédité «relativement» liée au sexe: l' X et l' Y sont constitués d'un segment pair, homologue pour les deux hétérochromosomes (mêmes *loci*) et d'un segment impair, dit différentiel, particulier à chacun d'eux. Etant donné l'homologie des segments pairs, il peut y avoir, à leur niveau, échanges de gènes et formation de chiasmata (Fig. 2).

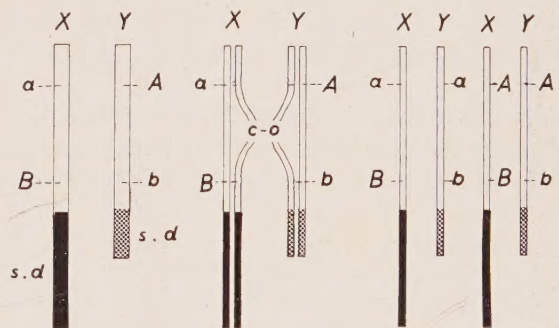


Fig. 2. L'hérédité relativement liée au sexe: au niveau du segment pair (en blanc) des $c-o$ sont possibles. Seuls les gènes situés sur les segments différentiels (en noir pour l' X , en quadrillé pour l' Y) sont strictement liés au sexe.

Le gène récessif b ne sera plus, après $c-o$, lié au chromosome Y mais au chromosome X ; il n'est donc plus que relativement lié au sexe. Par contre, les facteurs sis dans les segments différentiels demeurent rigoureusement liés à l' X ou à l' Y qui les portent.

Il est clair que, d'une espèce à une autre, ce mécanisme hypothétique aboutira à des résultats différents,

¹ K. OGUMA, *Cytologia* 5, 460 (1934).

² C. D. DARLINGTON, J. B. S. HALDANE et P. C. KOLLER, *Nature* 133, 417 (1934).

* Institut de Zoologie et d'Anatomie comparée.

selon les positions relatives des segments pairs et impairs et leurs rapports spatiaux avec le centromère. A titre d'exemple, voici comment, selon KOLLER et DARLINGTON³ (1934), le complexe sexuel du *Rattus norvegicus* doit être interprété (Fig. 3).

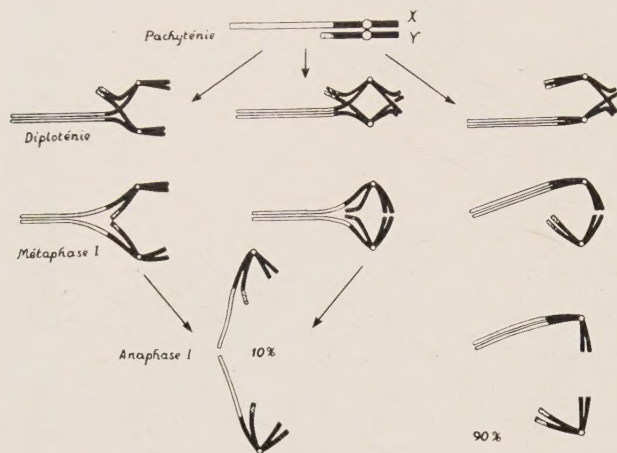


Fig. 3. La mécanique des chromosomes sexuels du Rat (*Rattus norvegicus*), d'après KOLLER et DARLINGTON³ (1934).

Cette conception a connu un grand succès auprès des généticiens et de nombreux travaux s'en inspirent. Peut-être est-il utile qu'un cytologiste mette les généticiens en garde contre la conviction que les bases cytologiques posées par KOLLER et DARLINGTON³ sont solides. C'est dans ce propos que, délaissant complètement l'aspect génétique du problème, je me limiterai à l'examen critique, selon des critères purement cytologiques, de l'hypothèse d'une hérédité relativement liée au sexe.

A la suite du travail consacré à *Rattus norvegicus* et exécuté en collaboration avec DARLINGTON, KOLLER (1936–1941) a étudié toute une série de mammifères, soit *Sciurus carolinensis*, *Talpa europaea*, *Putorius furo*, *Homo sapiens*, *Cricetus auratus*, *Felis domestica*, *Apodemus sylvaticus*, *A. hebridensis*⁴. AHMED étendit l'enquête à *Ovis aries* (1940)⁵ et PONTECORVO à *Cricetulus griseus* (1941)⁶. Dans tous les cas, ces auteurs firent des observations en bon accord avec l'hypothèse, laquelle prenait ainsi le caractère d'une véritable théorie. En passant, notons que cette théorie avait entre autres le gros avantage de replacer dans un cadre général le cas particulier des chromosomes sexuels et d'expliquer l'association préméiotique de l'X et de l'Y par le mécanisme chiasmatique habituel. Une conséquence importante de la conception est à souligner: lorsque le centromère est situé dans le segment pair (Fig. 3), il y aura à l'anaphase I, tantôt préréduc-

tion, tantôt postréduction des hétérochromosomes; on observe, en d'autres termes, une postréduction (ou, dans le cas des *Apodemus* une préréduction) facultative. KOLLER a donné des chiffres extraordinairement précis de ces pourcentages qui lui apparaissent constants pour une espèce donnée. De cette constance, il déduit, par une argumentation de probabilité, que la position du centromère peut être calculée. Cette introduction du quantitatif dans le domaine de la cytologie devait évidemment séduire les esprits par son caractère apparent de précision.

*

A l'époque où paraissent les travaux précités qui marquent l'apparition de l'école de DARLINGTON dans la cytologie mammalienne, il existait dans le monde deux groupes de chercheurs depuis longtemps spécialisés dans l'étude des chromosomes, chez les vertébrés en général, chez les mammifères en particulier: au Japon, dès 1928, MINOUCHI⁷ avait mis au point une méthode de fixation remarquable qu'OGUMA, puis surtout MAKINO, devaient exploiter au maximum. En Suisse, l'auteur de ces lignes avait abordé en 1927 l'étude cytologique des reptiles et, à partir de 1929, avait utilisé la méthode de MINOUCHI, ses investigations et celles de ses élèves suivant un cours parallèle à celui des recherches de l'école japonaise. Il était donc naturel que, dans l'étude critique des conceptions de KOLLER, ce soient les noms de MAKINO et de MATTHEY qui reviennent constamment, de 1934 à 1952. A partir de cette date, la création de nouvelles techniques rendant le travail beaucoup plus facile a provoqué l'apparition de nombreux chercheurs dans le domaine de la cytologie des mammifères.

Il est en effet évident que la valeur d'un travail cytologique dépend avant tout de la qualité du matériel étudié, donc de la technique de fixation. Je résumerai en un bref tableau quelles sont ces techniques et quelle appréciation chacune d'elle mérite lorsqu'elles sont appliquées au testicule des mammifères.

On voit, par ces données, que, de 1934 à 1950, la discussion cytologique s'est fondée à peu près uniquement sur l'analyse des coupes. Deux problèmes essentiels sont au centre de cette discussion: 1° une même espèce montre-t-elle à la fois de la préréduction et de la postréduction? 2° L'évolution préméiotique de l'X et de l'Y s'accompagne-t-elle d'une zygoténie prouvant l'existence d'un segment pair et autorisant des *c-o* et la formation de chiasmata?

*

MAKINO⁸ (1943) a d'emblée rejeté la conception nouvelle: «KOLLER and DARLINGTON emphasized that

³ P. C. KOLLER et C. D. DARLINGTON, J. Genet. 29, 159 (1934).

⁴ P. C. KOLLER, Proc. Roy. Soc. Ed. 56, 196 (1936); Proc. Roy. Soc. B. 121, 192 (1936); Proc. Roy. Soc. Ed. 57, 194 (1937); J. Genet. 36, 177 (1938); 61, 78 (1941); 41, 375 (1941).

⁵ I. A. AHMED, Proc. Roy. Soc. Ed. B. 61, 107 (1941).

⁶ G. PONTECORVO, Proc. Roy. Soc. Ed. 62, 32 (1943).

⁷ O. MINOUCHI, Jap. J. Zool. 1, 235 (1928).

⁸ S. MAKINO, J. Fac. Sci. Hokkaido Imp. Univ. 6, 9, 57 (1943).

Méthodes		
A) Le matériel est débité en coupes		
Fixateurs	Auteurs	Résultats
Bouin-Allen et variantes	PAINTER (1921) KOLLER <i>et al.</i>	Médiocres
Flemming acétique	WINIWARTER (1912)	Moyens
Champy ou Flemming sans acide acétique	MINOUCHI (1928) MAKINO MATTHEY <i>et al.</i>	Excellents mais inconstants
B) Le matériel est écrasé (<i>squashes</i>) ou, moins souvent, sert à la confection de frottis par apposition. Fixateurs à base d'acide acétique		
Pas de prétraitement préalable à la fixation	LA COUR (1944) SLIZYNSKI <i>et al.</i>	Médiocres à moyens
Traitement hypotonique préalable à la fixation	MAKINO et NISHIMURA (1952) HSU (1952) MATTHEY (dès 1953) WAHRMAN et ZAHAVI, FORD et HAMMERTON, TJIO et LEVAN <i>et al.</i>	Excellents et constants

the equational segregation of the X—Y chromosomes occurs in a few cases of the first division. The skillful observer may not doubt to see that the figures given by KOLLER and DARLINGTON... as the equational separation of the X—Y are nothing but the irregularly protruded chromosomes, caused by faulty fixation.» Disposant d'un matériel de haute qualité, MAKINO nie catégoriquement l'existence d'une zygoténie entre les hétérochromosomes, exclut donc toute formation de c—o ou de chiasmas et, sur 316 anaphases I, n'observe pas, chez le rat, un seul cas de postréduction. Chez la souris (1941), il arrive aux mêmes conclusions: l'X et l'Y n'entrent en contact qu'à la fin de la diploténie et par une extrémité seulement: 182 anaphases I ne montrent que la préréduction.

MATTHEY (*passim*, de 1936 à 1949) s'exprime d'une manière plus nuancée. Comme MAKINO, il critique la technique anglo-saxonne et se demande notamment comment les pourcentages extraordinairement précis de KOLLER ont pu être obtenus par l'analyse d'un matériel où le décompte brut des chromosomes n'était parfois pas possible: KOLLER (1938) n'a-t-il pas compté 38 chromosomes chez *Mesocricetus* au lieu de 44 (HUSTED, HOPKINS et MOORE⁹, 1945; MATTHEY¹⁰, 1952) et 38 chez *Talpa* (1936) au lieu de 34 (BOVEY¹¹, 1949)? PONTECORVO⁶ (1943) a dénombré 14 chromosomes chez *Cricetulus* au lieu de 22 (MATTHEY, 1952) et MULDAL¹² (1947) 46 éléments chez *Microtus agrestis* qui en pos-

sède 50 (MATTHEY¹³, 1949). Ces erreurs montrent bien que la technique de fixation est infidèle et très inférieure à celle qu'obtiennent MAKINO et MATTHEY. Mais, d'autre part, MATTHEY, comme le seront les généticiens, est impressionné par l'élégance de la solution proposée et par la grande autorité de DARLINGTON. Dans la série de ses travaux 1936–1949 et jusqu'en 1952, il s'efforcera de réunir des arguments en faveur de la théorie nouvelle plus encore qu'il ne la combattrà: en 1938¹⁴, dans un cas seulement, il pense observer une anaphase I de *Rattus* avec postréduction; il signale encore de la postréduction facultative chez *Arvicola* et de la préréduction facultative chez *Apodemus*; il reconnaît que KOLLER et DARLINGTON ont eu raison en montrant, contrairement à MAKINO et à MATTHEY, que le chromosome X de *Rattus* n'a pas un attachement terminal. Cependant, il ne peut jamais mettre en évidence une zygoténie préméiotique des hétérochromosomes et, sur ce point, ses observations sont en parfait accord avec celles de MAKINO. Au fur et à mesure qu'il étudie de nouvelles espèces, la fragilité de l'hypothèse lui apparaît plus grande: à l'époque où il écrit «*Les chromosomes des vertébrés*»¹⁵, il est partagé entre deux attitudes, comme en témoignent les deux citations suivantes: «Si l'on songe à l'importance prise en génétique humaine par la notion de segment pair et de caractères relativement liés au sexe on est quelque peu effrayé à la pensée que les généticiens se basent sur des notions que toute une école de cytologistes ... repous-

⁹ L. HUSTED, J. T. HOPKINS et M. B. MOORE, J. Hered. 36, 93 (1945).

¹⁰ R. MATTHEY, Chromosoma 5, 113 (1952).

¹¹ R. BOVEY, Rev. suisse Zool. 56, 371 (1949).

¹² S. MULDAL, J. INNES, Hort. Inst. 14, 19 (1949).

¹³ R. MATTHEY, Cellule, 53, 163 (1950).

¹⁴ R. MATTHEY, J. Genet. 36, 73 (1938).

¹⁵ R. MATTHEY, Les chromosomes des vertébrés (Lausanne 1949).

sent» ... «Cette belle découverte de KOLLER et DARLINGTON, bien que ses bases à proprement parler cytologiques soient fragiles ..., je me sens finalement enclin à l'admettre» (¹⁵, p. 132).

En 1952, le même auteur, après révision du cas de *Mesocricetus*, en arrive à la même conclusion que MAKINO, et cette conclusion a été citée par WHITE¹⁶ (1955) comme correspondant à sa propre conviction: «Si, en 1950, je me suis prononcé, bien qu'avec beaucoup de réserve, en faveur de la théorie de KOLLER et DARLINGTON, je suis de plus en plus enclin, devant l'accumulation des faits qui parlent contre elle, à la considérer comme une simple et ingénieuse vue de l'esprit».

Dès 1953, travaillant maintenant avec une technique sûre et rapide, (*vide supra*), MATTHEY¹⁷ accumule les observations: de 1953 à 1956, il étudie plus de 100 espèces de rongeurs, un prosimien, un insectivore. Les anciens cas douteux sont revus: il n'y a pas plus de préréduction chez les *Apodemus* que de postréduction chez les *Arvicola*. D'autre part, l'analyse faite en 1949 et 1950 de l'évolution préméiotique chez *Microtus agrestis*¹³, matériel très favorable en raison du gigantisme des hétérochromosomes, ne laisse aucun doute sur le fait que, de la léptoténie à la diacinèse, l'X et l'Y ne se présentent jamais sous la forme étirée qui autoriserait une zygoténie; il est vrai que l'opinion inverse a été soutenue par SACHS¹⁸ (1953), mais sans arguments sérieux. Le cercle va se refermer et le retour à MINOUCHI (1928!) se dessine ... SACHS¹⁹ (1954, 1955) réétudie la structure du bivalent sexuel chez l'homme, la souris et d'autres mammifères: l'hétéropycnose s'oppose à la formation de chiasmata qui, en fait, manquent complètement. TJIO et LEVAN²⁰ (1956) reprennent l'analyse de *Rattus* et leur description peut être superposée à celle de MINOUCHI; à la métaphase I, «the X and Y were always joined end to end. No chiasmata were ever seen».

*

Dès 1949, j'ai esquissé une interprétation de la post-réduction obligatoire des *Apodemus* en me fondant sur la notion d'«anticipation centromérique» que j'avais définie et développée au cours de l'analyse cytologique de la parthénogénèse chez une blatte, *Pycnoscelus surinamensis* (1945)²¹. Le partage équationnel de l'X et de l'Y à l'anaphase I, chez les *Apodemus*, implique une division précoce du centromère des hétérochromosomes; alors que, dans tous les autres cas, la division

du kinétochore ne deviendra effective qu'à l'anaphase II, elle intervient ici beaucoup plus tôt et représente la cause immédiate de la postréduction du complexe sexuel (Fig. 4).

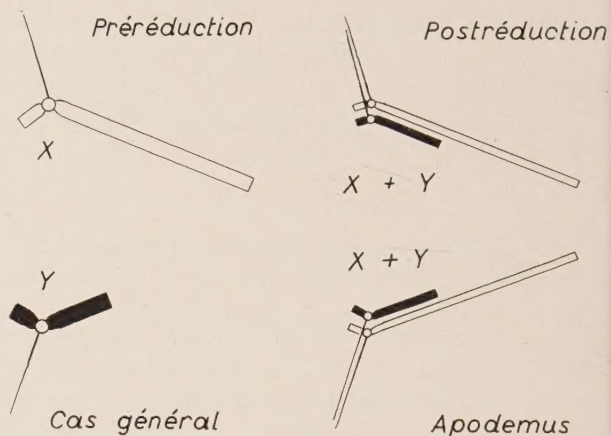


Fig. 4. Interprétation du cas général de la préréduction et du cas spécial d'*Apodemus* où l'anticipation centromérique détermine une postréduction (d'après MATTHEY¹⁵, 1949).

Chose curieuse, alors qu'il n'y a plus un seul argument d'ordre cytologique que l'école anglaise puisse tirer de ses propres travaux, on peut relever dans quelques uns de mes mémoires récents quelques observations à première vue favorable à l'idée d'échanges entre l'X et l'Y. Voici tout d'abord le cas de *Cricetus cricetus* et de *Cricetulus griseus* (Fig. 5).



Fig. 5. Liaison apparemment chiasmatique de l'X et de l'Y: A, B, C, chez *Cricetulus griseus*; D, chez *Cricetus cricetus* (MATTHEY²³, 1952).

Chez *Cricetulus*, de rares métaphases I montrent un chiasma très net entre l'X et l'Y, habituellement unis par leurs extrémités seules. Chez *Cricetus*, il semble également que c'est à un véritable chiasma que l'X et

¹⁶ M. J. D. WHITE, *Animal Cytology and Evolution* (Cambridge 1955).

¹⁷ R. MATTHEY, *Rev. suisse Zool.* 60, 225 (1953); *Caryologia* 6, 1 (1954); *Rev. suisse Zool.* 62, 163 (1955); 64, 39 (1957).

¹⁸ L. SACHS, *Heredity* 8, 117 (1953).

¹⁹ L. SACHS, *Ann. Eugen.* 18, 255 (1954); *Genetics* 27, 309 (1955).

²⁰ J. H. TJIO et A. LEVAN, *An. Est. exp. Aula Dei* 4, 173 (1956).

²¹ R. MATTHEY, *Rev. suisse Zool.* 52, 1 (1945).

l'Y doivent leur union. Ici encore, de telles figures sont exceptionnelles. Examinons maintenant la figure 6 qui

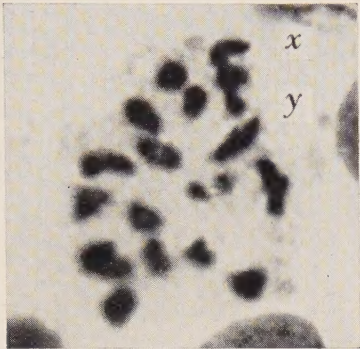


Fig. 6. Le complexe sexuel de *Leggada (Mus) minutoïdes* à la métaphase I (d'après MATTHEY, inédit, X 1.800).

se rapporte à la métaphase I de *Leggada minutoïdes* (MATTHEY, inédit). La présence d'un chiasma entre les hétérochromosomes est manifeste. Pour saisir ce que je pense être le sens véritable de ces observations, il est nécessaire de remettre ces cas très particuliers dans un cadre général: les divers types d'X et d'Y que j'ai rencontrés chez les Muridae sont représentés et classés dans la figure 7.

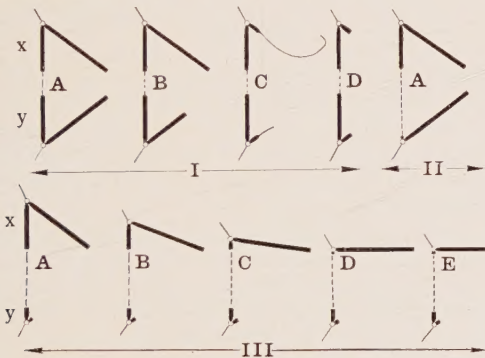


Fig. 7. Les types d'hétérochromosomes chez les Muridés (MATTHEY²³, 1954).

Nous voyons que les hétérochromosomes peuvent être presque homomorphes (I/A et I/B) ou très hétéromorphes (III/A – E). Entre ces extrêmes, tous les types de transition se rencontrent. Il est évidemment tentant d'admettre que l'homomorphie est primitive et que la différenciation de l'X et de l'Y s'est accentuée au cours de l'évolution. Il n'en est probablement pas ainsi: dans l'ensemble des mammifères, les types I et II sont très rares. Chez les Muridae, ils caractérisent quelques espèces de Murinae et de Microtinae, les trois genres étudiés de Cricetinae paléarctiques et les Gerbillinae dans leur ensemble, abstraction faite des espèces à chromosomes sexuels multiples, *Gerbillus pyramidum* et *G. gerbillus* (MATTHEY²², 1952, 1954; WAHRMAN et

ZAHAVI²³, 1955). Or, les rares cas de chiasmas observés entre l'X et l'Y l'ont été précisément chez des espèces de type I/C (*Cricetus*, *Cricetulus*) et I/B (*Leggada*).

Ce dernier cas me semble très révélateur: les *Leggada* sont placés par les systématiciens modernes (par exemple ELLERMAN, MORRISON-SCOTT et HAYMAN²⁴, 1953) dans le genre *Mus*, ce qui revient à admettre qu'elles sont très proches parentes des souris proprement dites. Comparons alors l'assortiment chromosomique de *Mus* et de *Leggada* (Fig. 8).

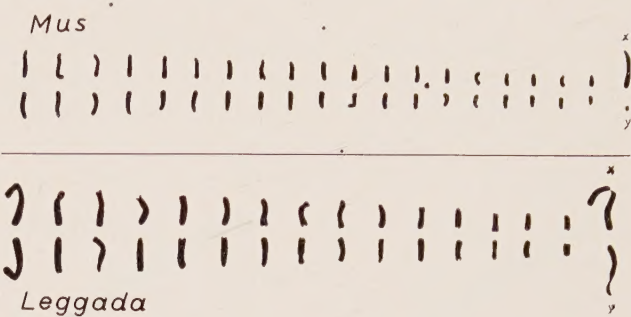


Fig. 8. La sériation des chromosomes chez *Mus musculus spretus* (MATTHEY¹⁷, 1955) et *Leggada (Mus) musculoïdes* (MATTHEY, inédit).

Chez sept espèces ou sous-espèces de *Mus*, nous trouvons le même assortiment chromosomique: les 40 chromosomes sont acrocentriques ou même télocentriques. L'X est le plus long et l'Y est le plus court. Ici, deux remarques: 1° SLIZYNSKI²⁵ (1948) n'a pas su identifier la position des centomères et dans sa «preliminary pachytene chromosome map» si souvent citée, presque tous les éléments sont submétacentriques ou métacentriques; une telle accumulation d'erreurs à un niveau où l'observation est relativement facile, fait planer quelques doutes sur la validité de la description relative à la structure fine des chromosomes et à la succession des chromomères; 2° TJIO et LEVAN²⁶ (1954) ont présenté de superbes figures et ont mis en évidence d'une manière particulièrement claire, la position terminale ou subterminale de tous les centomères. Par contre, ils n'ont pas su identifier correctement les chromosomes sexuels qui, selon eux, seraient à rechercher parmi les éléments de taille intermédiaire. Or, dès 1941, MAKINO²⁷ avait montré que l'X est plus long ou aussi long que les plus grands autosomes, alors que l'Y est plus court ou aussi court que les autosomes les plus petits. L'identification est plus facile encore dans la sous-espèce *Mus musculus spretus* (MATTHEY¹⁷, 1955) où l'X est spécialement long et l'Y particulièrement bref.

²² R. MATTHEY, Arch. Klaus-Stift. Vererb. Forsch. 27, 163 (1952); Exper. 10, 464 (1954).

²³ J. WAHRMAN et A. ZAHAVI, Nature 175, 600 (1955).

²⁴ J. R. ELLERMAN, T. C. S. MORRISON-SCOTT et R. W. HAYMAN, Southern african mammals (London 1953).

²⁵ B. M. SLIZYNSKI, J. Genet. 49, 242 (1948).

²⁶ J. H. TJIO et A. LEVAN, Lunds Univ. Arssk. 50, 1 (1954).

²⁷ S. MAKINO, J. Fac. Sci. Hokkaido Imp. Univ. 7, 305 (1941).

Chez *Leggada*, il n'y a que 32 chromosomes; une grande paire autosomique est métacentrique, les autres autosomes acrocentriques ou télocentriques; le fait surprenant c'est que l'*X* et l'*Y* sont de type *I/B*! (Fig. 6 et 8).

En comparant les nombres fondamentaux (nombre des bras principaux), nous arrivons aux chiffres suivants:

<i>Mus</i>	$2N = 40$	$N.F. = 40$
<i>Leggada</i>	$2N = 32$	$N.F. = 36$ ou 38 .

Il y a donc un déficit de «bras» chez *Leggada*, dont je propose l'explication suivante: l'*X* et l'*Y* de type *Mus* ont été transloqués sur une paire autosomique, selon le schéma suivant (Fig. 9):

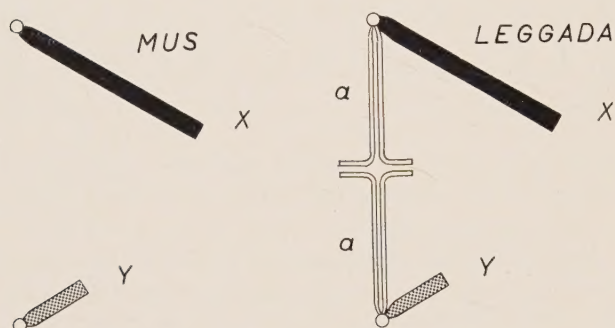


Fig. 9. L'interprétation des hétérochromosomes de *Leggada*: l'*X* et l'*Y* primitifs seraient transloqués sur une paire d'autosomes (*a*).

La portion autosomique n'apparaissant probablement pas dans les stades préméiotiques, on peut supposer que, lorsqu'une translocation est très ancienne, la faculté d'échanger des segments par *c-o* diminue peu à peu, la portion autosomique étant progressivement envahie par l'hétérochromatie des chromosomes sexuels proprement dits. Chez les *Leggada*, de telles figures sont très exceptionnelles, comme chez les *Cricetinae* et je n'en ai pas retrouvé l'équivalent chez les *Gerbillinae* et les *Microtinae* de type *I* où dans la liaison des hétérochromosomes interviennent seules les extrémités. Si cette hypothèse est plausible, elle sauverait l'«existence cytologique» du segment pair là où les chromosomes sexuels ont été transloqués sur une paire d'autosomes, c'est-à-dire dans des cas très rares. Si elle ne l'est pas, la signification des chiasmas observés demeure énigmatique mais ces quelques observations, si certaines soient-elles, ne sauraient suffire à conserver la notion d'une hérédité relativement liée au

sexe qui, sur le plan cytologique, est condamnée par l'ensemble des observations que j'ai présentées ici.

*

Dans une discussion figurant à la suite d'une note récente de FORD et HAMERTON²⁸ (1956), le professeur KOLLER s'exprime de la façon suivante: «As regards partial sex-linkage, we must admit that there is no cytological proof...» et le Dr C. E. FORD «agrees with Dr. SACHS that the evidence in favour of partial sex-linkage is now very slender...».

Pour moi, je ne puis m'empêcher de constater que, faute d'avoir pris en suffisante considération la littérature étrangère, l'école anglaise redécouvre maintenant ce que MAKINO et moi-même avons démontré depuis longtemps. En guise de conclusion, je transcris ces lignes empruntées à un travail de 1939²⁹ et qui n'ont rien perdu de leur actualité: «... il faut savoir oublier la Génétique et ses données, si nous – cytologistes – voulons éviter l'asservissement de notre discipline condamnée de plus en plus à toujours découvrir, si obscurs que soient les faits, une docile explication pour tous les résultats expérimentaux des généticiens».

Summary

The author shows that the conception of an incomplete sex-linkage in mammals must be discarded on cytological grounds. There is either (general case) pre-reduction of the *X* and *Y* chromosomes at the first metaphase, or post-reduction (*Apodemus*) but never facultative post- or pre-reduction.

During the growth period, from the Zygoteny to the Diacinesis, the occurrence of *c-o* is made highly improbable, owing to the heteropycnotic state of the sex-chromosomes, and has never been observed with accuracy. The revision of the literature shows that such an hypothesis is not tenable.

The obligatory post-reduction by *Apodemus* can be interpreted in the concept of 'anticipation centromérique'.

In a very few cases, formations like chiasmas occur as linking the *X* and *Y* chromosomes at the first metaphase. It is possible that these exceptional configurations might be explained by a translocation of the sex-chromosomes on a pair of autosomes.

From a more general standpoint, it would be desirable that geneticists should have more confidence in the results of pure cytologists rather than in the work of cytogeneticists who wish to find an explanation for every genetical hypothesis.

²⁸ C. E. FORD, A. Genet. Stat. Med. 6, 264 (1956).

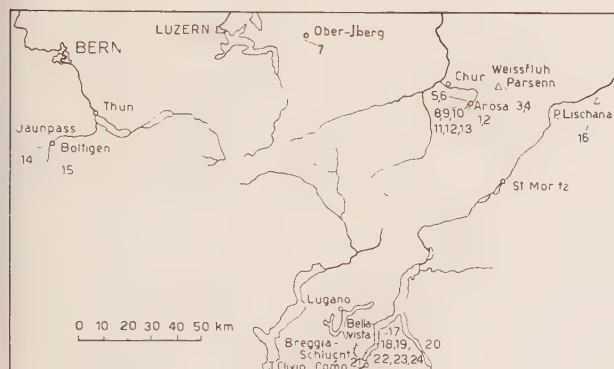
²⁹ R. MATTHEY, Arch. Biol. 50, 431 (1939).

Brèves communications - Kurze Mitteilungen
Brevi comunicazioni - Brief Reports

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Spurenelemente einiger schweizerischer Ophiolithe, Radiolarite und Mergel

1. *Einleitung.* Die Untersuchungen eines der Verfasser (GRUNAU¹) über Probleme der Farbschichtung ergaben, dass die Grünfärbung alpiner Radiolarite in gewissen Fällen durch ein feinverteiltes Pigment ophiolithischer Herkunft bedingt ist. Zur weiteren Abklärung dieser Frage wurde die Bestimmung des Spurenelementgehaltes (HÜGI) von 24 Gesteinsproben (siehe Fig. und Tab. I–III) veranlasst. Dabei liess sich vermuten, der Spurenelementgehalt grüngefärbter Radiolarite müsse qualitativ analog sein wie in gleichaltrigen oder älteren Ophiolithen, quantitativ jedoch infolge starker «Verdünnung» bedeutend geringer, wenn der Grünfärbung Serpentin- oder Peridotit-Partikel zugrunde liegen.



Fundortskizze der analysierten Proben. Die Nummern beziehen sich auf Tabellen I–III.

2. *Methodisches.* Von den Handstücken wurden Durchschnittsproben entnommen und mittels eines Zeiss-Quarzspektrographen (Modell Q 24) nach der Glimmschichtmethode im Gleichstromkohlebogen analysiert (9 A, 80 V). Die Auswertung der Spektrogramme ergab die auf Tabellen I–III dargestellten Spuren-Elemente. In grösseren Mengen (meist als Hauptbestandteil) waren Elemente wie Al, Ca, Fe und Si vorhanden. Die Elemente Ag, Cd, Sc, Sn und W sind entweder nicht anwesend oder treten bloss in Mengen auf, die unterhalb der Nachweisempfindlichkeitsgrenze liegen. Die Nachweisempfindlichkeitsgrenze liegt für Ba bei 5 p.p.m., Cr 1, Cu 10, Ga 1, Mn 1, Ni 3, Pb 10, Sr 3, V 1.

3. *Ergebnisse.* Tabelle I zeigt den Spurenelementgehalt einiger Ophiolithe. In Serpentin, Peridotiten und Spiliten verhalten sich die Werte für Cu und Cr ziemlich konstant (0,01 bis höchstens 1%). Gewissen Schwankungen ist der Mn-Gehalt unterworfen, der allerdings wegen des zu geringen Auflösungsvermögens des Spektrographen und Eisenreichtums zum Teil nicht erfassbar ist. Ni ist in Serpentin und Peridotit im Ver-

gleich zu Spilit relativ angereichert, Spilite hingegen zeigen hohen Ti-Gehalt.

Tabelle 1. Spurenelemente einiger Ophiolithe.

	1	2	3	4	5	6	7
Ba	<i>st</i>	<i>m-st</i>	<i>m</i>	<i>m</i>	<i>m</i>	<i>s-m</i>	<i>m</i>
Cr	<i>m</i>	<i>m</i>	<i>m</i> (+)	<i>m</i> (+)	<i>m</i>	<i>s-m</i>	<i>m</i>
Cu	<i>m-st</i>	<i>m</i> (+)	<i>m</i>	<i>m</i>	<i>m</i>	<i>m</i>	<i>m</i>
Ga	—	—	—	—	<i>s</i>	<i>s</i>	<i>s</i>
Mn	<i>m</i>	<i>m</i>	?	?	<i>s</i> ¹	<i>m</i> ²	<i>s-m</i>
Ni	<i>m</i>	<i>s-m</i>	<i>s-m</i>	<i>s-m</i>	<i>s</i>	<i>s</i>	<i>s</i>
Sr	<i>m</i>	<i>s-m</i>	<i>s-m</i>	<i>s-m</i>	<i>s-m</i> ³	<i>s</i> ⁴	<i>s</i>
Ti	<i>m</i>	<i>m</i>	<i>s-m</i>	<i>s-m</i>	<i>st</i> ⁵	<i>st</i> ⁶	<i>st</i>
V	<i>s</i>	<i>s</i>	<i>s</i> ?	<i>s</i> ?	<i>s</i> ?	— (?)	<i>s</i> ?

¹ MnO = 0,19%
$$^2 \text{ MnO} = 0,28\%$$
GRUNAU²³ 220 p.p.m. Sr

4 94 p.p.m. Sr

TUREKIAN U. I.

⁵ TiO₂ = 2,05%
$$^6 \text{TiO}_2 = 4,27\%$$

GRUNAU²

1 Serpentin, Arosa

2 Serpentin, Arosa

3 Peridotit, Parsennfurka

4 Peridotit, Weissfluh-Parsenn

5 Spilit (intersertal), Hörnli bei Arosa

6 Spilit (variolitisch), Hörnli bei Arosa

7 Spilit, Ober-Iberg (Kt. Schwyz).

Richtwerte: $ss \ll 0,001-0,01\%$; $s \lesssim 0,01\%$; $m \gtrsim 0,01\%$;
 $st/stst$ um 1% und mehr.

In Serpentin und Peridotiten ist Cr an Picotit, untergeordnet auch an Olivin und Pyroxen, Cu an Kupferkies und Titan an Titanit, Leukoxen und Rutil gebunden (GRUNAU², GEES⁴). Dünnschliffe von Spiliten zeigen folgende Titanminerale: Titanit, Ilmenit, Leukoxen, Rutil und Anatas (GRUNAU²). Cr- und Cu-Mineralien wurden mikroskopisch nicht erkannt. Cr ist vermutlich an Pyroxene gebunden.

Ein deutlicher Unterschied im Spurenelementgehalt zwischen Serpentin und Peridotiten einerseits und Spiliten andererseits zeigt sich bloss beim Titan und, viel schwächer, beim Nickel.

Der Spurenelementgehalt einiger Radiolarite ist auf Tabelle II ersichtlich. Einen schwachen Cr-Prozentsatz besitzen bloss Radiolarite aus der Aroser Zone, aus dem Simmental und aus dem Unterengadin (Piz Lischana), während die Südtessiner Radiolarite Cr-frei sind. Mn ist sowohl in den ost- wie in den südalpinen Hornsteinen vertreten (es wurden bloss Proben untersucht, die makroskopisch keine Mn-Erze erkennen lassen). In ostalpinen Radiolariten ist der Ti-Gehalt im Vergleich zu südalpinen Hornsteinen relativ bedeutender. Der Cu-Wert ist überall ziemlich konstant, der Ba-Gehalt (*m* bis *st*) auffallend hoch. Eine Ausnahmestellung nimmt der grügraue Radiolarit von Rothried bei Boltigen

² H. GRUNAU, Diss. Bern 1947, p. 63.

³ K. K. TUREKIAN und J. L. KULP, *Geochim. cosmochim. Acta* 10, 248 (1956).

⁴ R. GEES, Schweiz. min. petrogr. Mitt. 36, 457 (1956).

¹ H. GRUNAU, *Eclogae geol. Helv.* 49, 505 (1957).

Tabelle II. Spurenelemente einiger Radiolarite.

	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Ba	st	m-st	m	m-st	m	m	st	st	st	st-stst	m	st	m	st
Cr	—	s	—	—	—	s-m	ss	s	s-m	—	—	ss?	—	—
Cu	m	m	m	m	s-m	m	s-m	m-st	m	s	s-m	s-m	s-m	m
Ga	—	ss	—	ss	—	ss	ss	ss-s	ss-s	ss?	ss	ss?	—	—
Mg	st	st	stst	stst	stst	stst	st-stst	stst	stst	stst	stst	stst	stst	st
Mn	s-m	s-m	m	s-m	s	m	m	m	m	m	s	m	m	s-m
Ni	—	s	ss?	—	—	—	ss-s	s-m	s	ss	—	ss?	—	—
Pb	—	—	—	—	—	—	ss	s	—	—	ss	—	—	—
Sr	m	s-m	s	s-m	m	s	m	st	m	m	m	m	s	m
Ti	s	s-m	s	—	—	m	m	m	m(+)	—	—	s	s	s
V	—	—	—	—	—	—	s	s	—	—	—	—	—	—

- 8 grüngrauer und rotbrauner Radiolarit, Arosa;

9 grüngrauer und grauroter Radiolarit, Arosa;

10 grüngrauer Radiolarit, Arosa;

11 grüngrauer Radiolarit, Arosa;

12 grüngrauer Radiolarit, Arosa;

13 rotbrauner Kieselchiefer, Arosa;

14 grüngrauer und rotbrauner Radiolarit, Jaunpass (Kt. Bern);

15 grüngrauer Radiolarit, Rothried bei Boltigen, Simmental (Kanton Bern);
- 16 grüngrauer Radiolarit, P. Lischana (Unterengadin, Graubünden);

17 lichtgrauer Radiolarit, Bellavista (Süd-Tessin);

18 grüngrauer Radiolarit, Breggiaschlucht (Süd-Tessin);

19 rotbrauner Radiolarit, Breggiaschlucht (Süd-Tessin);

20 rotbrauner Radiolarit, Breggiaschlucht (Süd-Tessin);

21 rotbrauner Radiolarit, T. Clivio (Oberitalien nahe Schweizer Grenze bei Arzo).

(Simmen-Decke) ein. Verglichen mit Radiolariten aus anderen tektonischen Einheiten sind in ihm Cu, Pb und Sr angereichert. Ein ähnliches gilt, wenn auch in etwas schwächerem Masse, für den Radiolarit der Jaunpass-Strasse (Simmen-Decke).

Auf Grund des Spurenelementgehaltes lassen sich drei Bezirke ausscheiden, die gleichzeitig auch mit tektonischen Einheiten zusammenfallen: 1. Der ostalpine Radiolaritbezirk (Cr und Ti relativ angereichert). 2. Der südalpine Radiolaritbezirk (Cr-frei und Ti-arm). 3. Der Radiolaritbezirk der Simmen-Decke im Gebiete des Simmentals (Cu, Pb und Sr relativ angereichert).

Tabelle III. Spurenelemente einiger Mergel.

	22	23	24
Ba	st	st	st-stst
Cr	s-m	—(ss?)	ss-s
Cu	m-st	m	s
Fe	stst	st-stst	stst
Ga	—	s	ss
Mg	stst	stst	stst
Mn	m ¹	m ²	m ³
Ni	m	—	s
Pb	—	s	s
Sr	m	m	m
Ti	m	m	m
V	m	—	s

¹ MnO = 0,18% ² MnO = 0,18% ³ MnO = 0,16%

- 22 dunkelgrauer, bituminöser Fischechiefer aus der Buntén Scaglia (Breggiaschlucht, Süd-Tessin);

23 graugrüner Mergel aus der Buntén Scaglia (Breggiaschlucht, Süd-Tessin);

24 rotbrauner Mergel aus der Buntén Scaglia (Breggiaschlucht, Süd-Tessin).

Als neutrale Vergleichsbasis liegen die Spurenelementgehalte von 3 oberkretazischen Mergelproben aus der Buntén Scaglia der Breggia-Schlucht im Süd-Tessin vor (Tab. III). Eine Ausnahmestellung nimmt der dunkelgraue, bituminöse Mergel (sog. Fischechiefer, VONDERSCHMITT⁵) ein, in dem Cr, Cu, Ni und V relativ angereichert sind. Bei dem hohen Schwefelgehalt dieses Mergels (S = 1,93%) ist die Konzentration bestimmter Schwermetallsulfide durchaus verständlich. Alle drei Proben weisen einen konstanten Mn- und Ti-Gehalt auf.

Vergleich der erhaltenen Werte. Zunächst interessiert das Verhältnis von Ophiolithen zu Radiolariten. Wie erwartet, zeigt sich beim Cr-Gehalt qualitativ eine gute Übereinstimmung zwischen Ophiolithen und Radiolariten des Ostalpins und der Simmen-Decke. Quantitativ lässt sich in den Radiolariten, bei denen die ophiolithischen Farbpartikel bloss wenige Prozente des Gesamtgesteinsvolumens ausmachen, eine «Verdünnung» und somit ein niedriger Cr-Gehalt erkennen. Dünn-schliffbeobachtungen zeigen zudem, dass Picotitpartikel sehr regellos und in geringer Häufigkeit in Radiolariten auftreten. Ein gleiches wie für Cr gilt für Ti, in schwächerem Ausmass auch für Ni. Eine Sonderstellung nehmen hingegen die Cr-freien und Ti-armen Südtessiner Radiolarite ein.

Der Mn- und Sr-Gehalt der analysierten Radiolarite und Ophiolithe ist ziemlich konstant.

Die Spurenelementgehalte der Radiolarite sowie des grüngrauen und roten Mergels der Buntén Scaglia verhalten sich, mit Ausnahme des Cr-Wertes, überraschend ähnlich. Eine Sonderstellung kommt bloss dem bituminösen Mergel zu.

4. Geologische Deutung. Spurenelement- und Dünn-schliffuntersuchungen alpiner Radiolarite und Ophiolithe ergeben folgende Gesichtspunkte:

a) Im oberjurassischen Sedimentationstrog der Arosar Zone Graubündens ist die Grünfärbung der Radiolarite und begleitenden Kieselchiefer auf ein feinverteilt

⁵ L. VONDERSCHMITT, Eclogae geol. Helv. 33, 212 (1941).

tes Pigment ophiolithischer Herkunft zurückzuführen. Als Materialspender fallen Serpentine und Peridotite in Betracht, deren Förderung syn- oder praeeoberjurassisch erfolgte (GRUNAU⁶, GEES⁴).

b) Die oberjurassischen Südtessiner Radiolarite zeichnen sich aus durch das Fehlen typisch lauchgrüner Farbtöne, durch einen Cr-freien und Ti-armen Spurenelementbezirk und einen beträchtlichen Karbonatanteil. Ein direkter oder indirekter Ophiolitheinfluss hat sich hier nicht bemerkbar gemacht.

c) Die Grünfärbung einiger oberjurassischer Radiolarite der Simmen-Decke ist durch ein Farbpigment unbekannter Herkunft bedingt. Es wäre vielleicht zu denken an ein prae-triasisches, kristallines Liefergebiet oder an einen jurassischen Ophiolith-Anteil der Simmen-Decke, der allerdings heute vollständig wegerodiert ist. Die spärlichen Reste von Albit-Diabasen sind bekanntlich jünger als Cenoman und älter als Priabon (ARBENZ⁷, GRUNAU⁸) und stehen somit in keiner Beziehung zur Grünfärbung.

H. R. GRUNAU und TH. HÜGI

Geologisches Institut und Mineralogisch-Petrographisches Institut der Universität Bern, den 2. Mai 1957.

Summary

The trace element content of a few Swiss ophiolites, radiolarian cherts and marls has been determined by means of spectrographic methods.

Field observations, microscopical and spectrographical data lead to the conclusion that the colour of green cherts in certain areas is due to the supply of clastic peridotite- and serpentine-particles originating from a syn-temporaneous ultrabasic igneous body.

⁶ H. GRUNAU, *Eclogae geol. Helv.* 39, 258 (1947).

⁷ K. ARBENZ, *Beitr. geol. Karte Schweiz [N.F.]* 89, 41 (1947).

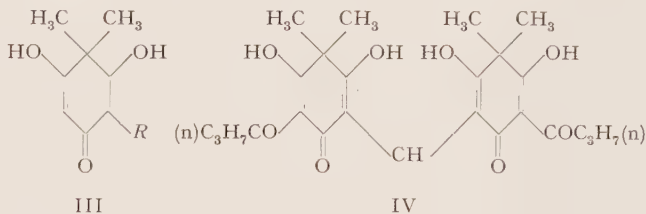
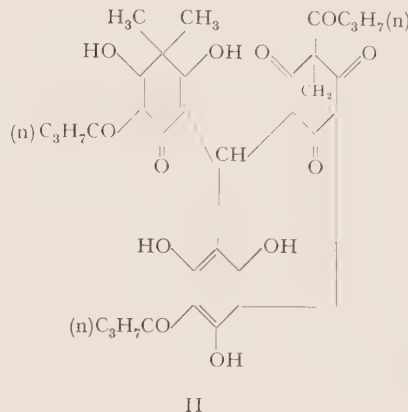
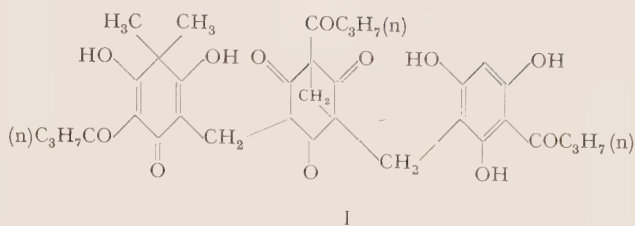
⁸ H. GRUNAU, *Schweiz. min. petrogr. Mitt.* 25, 325 (1945).

The Constitution of Filixic Acid

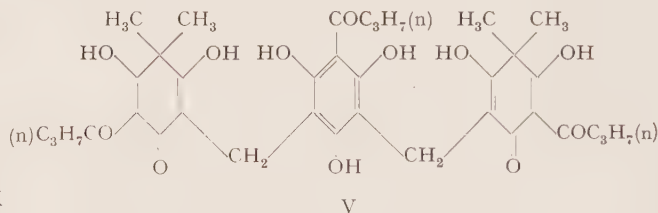
BOEHM¹ has proposed the alternative structures I and II for filixic acid, a major, biologically-active constituent of the resin of *Aspidium Filix mas*. These structures were based largely on the fact that filixic acid is hydrolysed by zinc dust and alkali to a mixture of butyric acid, filicinic acid (III, $R = H$) 1:3:5-trimethylphloroglucinol and *n*-butanoylfilicinic acid (III, $R = -COCH_2CH_2CH_3$). The yields of the filicinic acids produced on hydrolysis indicated that at least two filicinic acid moieties were present in the filixic acid molecule.

Also, albaspidin (IV) was obtained when filixic acid was refluxed for a prolonged time with ethanol. *Bis*-benzene-azophlorobutyrophenone was formed when filixic acid was treated with diazoaminobenzene. These degradation experiments suggested that filixic acid contained two *n*-butanoylfilicinic acid units, as in albaspidin, combined with a single phlorobutyrophenone unit. In order to account for the formation of 1:3:5-trimethylphloroglucinol in the reductive hydrolysis, structures were proposed in which more than one methylene bridge connected particular nuclei.

¹ R. BOEHM, *Liebigs Ann. Chem.* 318, 253 (1901).



We suggest the alternative structure V for filixic acid.



This indicates a molecular formula $C_{36}H_{44}O_{12}$ (required: C, 64.6; H, 6.63; O, 28.71) which agrees well with our experimental results (found: C, 64.6; H, 6.50; O, 29.00) and those of BOEHM, but cannot be distinguished with certainty by this means from the formulae $C_{35}H_{40}O_{12}$ (required: C, 64.4; H, 6.14; O, 29.46) proposed by BOEHM. On the basis of the structure V the formation of 1:3:5-trimethylphloroglucinol is to be attributed to a disproportionation reaction² similar to that which leads to the formation of 1:3:5-trimethylphloroglucinol from flavaspodic acid³. Similarly, it has been shown that albaspidin may be formed from flavaspodic acid by disproportionation in mild alkaline conditions³. This provides an explanation for the formation of albaspidin from filixic acid that does not require that the two butanoylfilicinic acid units in filixic acid should be in adjacent positions.

² A. J. BIRCH, *J. chem. Soc.* 1951, 3026.

³ A. MCGOOKIN, A. ROBERTSON, and T. H. SIMPSON, *J. chem. Soc.* 1953, 1828.

The ultraviolet absorption spectrum of filixic acid is in agreement with structure V. In ethanol it has maxima at 228 m μ (ϵ 41,000) and 288 m μ (ϵ 29,000) which are typical of enolised 2-acylcyclohexane-1:3-diones⁴. In connection with studies on the constitution of aspidin and flavaspidic acid AEBI⁵ has shown that the extinction values of these compounds may be arrived at by addition of the extinction values of the component nuclei insulated by methylene groups. The addition of the extinction values at the absorption maxima of two butanoylfilixic acid nuclei ($2 \times 12,510$) and one phlorobutyrophenone (ϵ 13,000) in the region of 228 m μ gives a value of 38,120 which is in reasonable agreement with the observed value. Similarly, the calculated value at the higher wavelength maximum is 27,210.

Filixic acid does not give a coloration with GIBBS 2:6-dichloroquinonechloroimide reagent⁶. This makes suggest substitution of unlikely alternative structures for filixic acid in which there are unsubstituted positions *para* to phenolic hydroxyl groups.

W. R. CHAN* and C. H. HASSALL

Department of Chemistry, University College of Swansea (Wales), April 16, 1957.

Zusammenfassung

Es wird die Vermutung ausgesprochen, dass Filixsäure, ein Inhaltsstoff von Farnwurzeln, die Konstitutionsformel (V) hat.

⁴ W. R. CHAN and C. H. HASSALL, J. chem. Soc. 1956, 3495.

⁵ A. AEBI, Helv. chim. Acta 39, 153 (1956).

⁶ H. D. GIBBS, J. biol. Chem. 72, 649 (1927).

* Present address: Department of Chemistry, University of Glasgow.

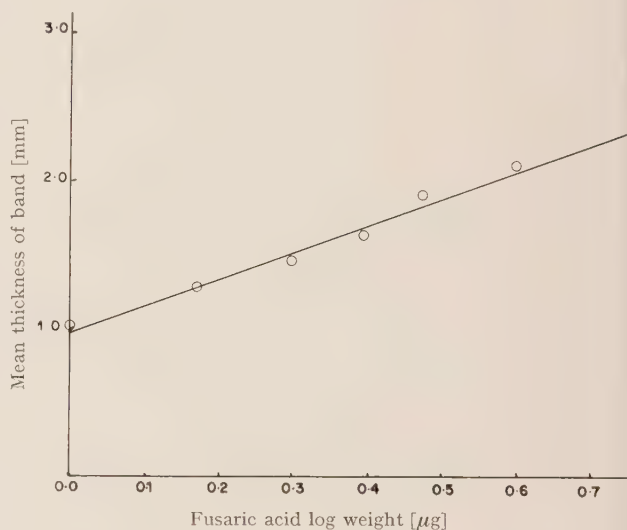
Chromatographic Detection and Estimation of Fusaric Acid

Fusaric acid was first isolated by GÄUMANN and his school of workers¹ from cultures of *Fusarium lycopersici* Sacc. and has since been known to be produced by several Fusaria. The presence of this toxin *in vivo* in wilt-infected cotton plants was reported by us earlier² and established to be the *vivo*-toxin in the *Fusarium* wilt of cotton. ZAHNER³ reported the chromatographic detection and bio-assay of fusaric acid; and KALYANASUNDARAM⁴, in this laboratory, developed the agar-cup technique for its assay using a soil bacterium. In view of the considerable difficulty in obtaining sharp demarcation of the fusaric acid band separated chromatographically, possibly owing to the poor ionization of the acid, we have earlier indicated the advantages of separating and identifying this toxin as its copper chelate⁵. In the present communication the detection and quantitative estimation of fusaric acid is described with the technique of paper disk chromatography developed by one of us⁵. It was considered desirable to adapt the

area method developed by FISHER *et al.*⁶ in preference to elution methods which have practical limitations in the small chromatograms especially when complexes with close Rf values are present.

The following amounts (as aqueous solution) of fusaric acid contained in 0.75 μ l were spotted in the centre of circular filter papers (7.4 cm diameter) followed by 4 μ g of copper as CuSO₄ (5 mg/ml): 1.0; 1.5; 2.0; 2.5; 3.0 and 4 μ g. The papers were air-dried and irrigated as indicated elsewhere⁵ controlling the period of irrigation at 95 min. The solvent used was *N*-butanol-acetic acid-water (4:1:5) and filter paper-Whatman No. 1. The irrigated chromatograms were sprayed with rubeanic acid (0.1% in acetone) and the colour of the bands intensified by exposure to ammonia vapour⁷ (10 s). The developed chromatograms were cut into regular octogens tangentially in the 8 directions ($\pm 45^\circ$), mounted between two square glass plates and sealed on two sides with paraffin wax. The thickness of the Cu-fusaric acid com-

Log fusaric acid – mean thickness curve *n*-butanol-HAC-water 4:1:5.



plex band was measured on the microscope using an ocular micrometre (low power). The stage readings were recorded between the points where the green colour appeared and disappeared. The mean thickness of the band in the 8 directions was computed (as the mean of 6 chromatograms) for each concentration (varying $\pm 3\%$) and plotted against log amount of the toxin on the abscissa. The typical standard curve obtained is shown in the Figure. It would be obvious that the mean thickness bears a linear relationship to the areas of the bands since the Rf values were consistent and reproducible and the bands quite circular. The areas of the complex bands are directly proportional to the logarithm of the amount of fusaric acid and independent of the total copper spotted when it is within reasonable limits. The quantitative estimation of fusaric acid in an unknown sample could easily be carried out by measuring the mean thickness of its Cu-chelate in the range of sensitivity and the amount read as the antilog of the abscissa. It may be added that the curve is not quite sensitive at concentrations of fusaric acid lower than 1 μ g or higher than 4 μ g.

¹ E. GÄUMANN *et al.*, Phytopath. Z. 20, 1 (1952).

² K. LAKSHMINARAYANAN and D. SUBRAMANIAN, Nature 176, 697 (1955).

³ H. ZAHNER, Phytopath. Z. 22, 227 (1954).

⁴ R. KALYANASUNDARAM, J. Indian bot. Soc. 34, 43 (1955).

⁵ K. LAKSHMINARAYANAN, Arch. Biochem. Biophys. 49, 396 (1954).

⁶ R. B. FISHER, D. S. PARSONS, and G. A. MORRISON, Nature 161, 764 (1948).

⁷ K. LAKSHMINARAYANAN, Proc. Indian Acad. Sci. 40 B, 167 (1954).

Employing the present technique, the fusaric acid content of the dialysed culture filtrate of *F. vasinfectum* Atk. (grown in Richard's medium in Haffkine flasks—1.5 l medium/4 l flasks for 8 weeks) was found to be 44.6 mg/l. The dialyzed culture filtrate was concentrated to a small volume under reduced pressure prior to spotting. The fusaric acid content was estimated as indicated earlier. The concentrate was then diluted to 75% and 50% (with distilled water) and spotted again. Pure fusaric acid was added to make up the difference in each case and the total fusaric acid content estimated for recovery. The recoveries obtained at the two levels were 95.5% and 99%, respectively indicating that the method could be employed with advantage for the assay of this toxin.

Rf values of copper and copper-fusaric acid complex in different solvent mixtures.

Solvent mixtures	Rf values	
	Copper	Complex
1. <i>N</i> -butanol-HAc-water (4:1:5)	0.494	0.273
2. <i>N</i> -butanol-HAc-water (3.5:1.5:5)	0.666	0.617
3. <i>N</i> -butanol-formic acid-water (5:3.5:5)	0.157	0.088
4. Isoamyl alcohol-HAc-water (4:1:5)	0.230	0.090
5. Isopropanol-formic acid-water (4:1:5)	0.460	0.400
6. Aq. Dioxan (1:1)	0.790	0.630
7. Aq. phenol	0.110	Not formed
8. 400 ml of aq. butanol containing 5 g 8-quinolinol to which was added 40 ml glacial acetic acid warmed to solution	0.460	0.180
9. Incorporation of 0.5 g EDTA* in 100 ml aq. butanol	0.100	Not formed
10. Impregnating the paper with 10% alumina prior to irrigation with <i>N</i> -butanol-HAc-water (4:1:5)	0.230	Not formed
11. Aq. Collidine	0.182	0.263
12. Aq. Lutidine	0.784	0.299

* Ethylene diamine tetracetate.

The Table gives the Rf values of copper and Cu-fusaric acid complex in the 12 solvent mixtures indicated therein. It may be added that the method could be employed only in the absence of interfering metabolites which would either alter the Rf value of the complex or form chelates having close Rf values. In such cases it would be necessary to use different solvents to eliminate interference and use the corresponding standard curves.

We thank Professor T. S. SADASIVAN for helpful suggestions, Professor E. GÄUMANN for a generous gift of pure fusaric acid and the National Institute of Sciences of India for the award of an I.C.I. fellowship to one of us (K.L.).

K. LAKSHMINARAYANAN* and
D. SUBRAMANIAN

University Botany Laboratory, Madras (India), April 4, 1957.

Zusammenfassung

Es werden der chromatographische Nachweis und die Bestimmung der Fusarinsäure als Kupferchelate mittels der Rundfiltertechnik beschrieben. Die durchschnittliche Breite des Chelatstreifens steht in einem linearen Verhältnis zu dem Logarithmus der Konzentration von Fusarinsäure. In einem Kulturfiltrat von *F. vasinfectum* Atk. war ein Zusatz von reiner Fusarinsäure quantitativ nachweisbar. Rf-Werte von Cu und Cu-Fusarinsäure-Komplexen in 12 Lösungsmitteln werden angegeben.

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Carbohydrates, Collagen and Elastin of the Normal Aortic Wall and Arteriosclerotic Hyaline Plaques

Reports in the literature on the determination of the carbohydrates and of the collagen and elastin content in the normal aorta and their possible modifications in the course of arteriosclerosis, are relatively few. However studies carried out by means of metachromatic stain reactions indicate that the elastic tissue fibers of the aorta are bathed in a matrix of 'ground substance' constituted mainly of sulphated acid mucopolysaccharides, and that the alterations of this mucinous substance could be followed by modifications of the content in collagenous and elastic fibres of the aorta, which may be of fundamental importance in the evolution of arteriosclerosis¹.

Therefore, in the present research the chemical composition of hyaline plaques from arteriosclerotic aortae was investigated and compared with that of a normal aortic wall.

The samples were severed in three fractions, labelled F. I.—F. II.—F. III.—, according to NEUMAN and LOGAN². We have analyzed samples of 9 different normal aortic walls of men aged from 20 to 40 years and samples collected from hyaline plaques of 9 different cases of arteriosclerosis; the amount of tissue, from each case, which was necessary for chemical analysis, was about 1 g wet weight.

The first fraction contains mainly collagen, the second fraction contains several heterogenous proteins, and the third one contains elastin. The nitrogen was determined in each fraction by Nesslerization³; in addition, the hydroxyproline content in F.I., in order to define its true collagen quota, was determined by the method of TROLL and CANNAN⁴. The carbohydrates were determined by the methods described by DISCHE⁵. The presence of the SO₄⁻ groups was investigated qualitatively by adding BaCl₂ 1% to the samples, hydrolized in 5 N HCl, dried and made up to ml 1 with water, in order to precipitate BaSO₄.

¹ J. F. RINEHART, in *Connective tissue in health and disease* (Munksgaard, Copenhagen 1954), p. 239.

² E. E. NEUMAN and M. A. LOGAN, *J. biol. Chem.* 186, 549 (1950).

³ W. W. UMBREIT, R. H. BURRIS, and J. F. STAUFFER, *Manometric techniques and tissue metabolism* (Burgess Pub., Minneapolis 1949).

⁴ W. TROLL and R. K. CANNAN, *J. biol. Chem.* 200, 803 (1953).

⁵ Z. DISCHE in *Methods of Biochemical Analysis* (Interscience Pub. Inc., New York 1955), Vol. II, p. 313.

		Normal aorta	Pathologic aorta	<i>t</i>	<i>P</i>
Protein .	F. I	49 ± 2	53 ± 4	0.918	> 0.05
Nitrogen }					
Collagen	F. I	18 ± 1.3	25.6 ± 3.1	2.478	< 0.05
Nitrogen }					
Protein .	F. II	20 ± 2	29 ± 5	1.738	> 0.05
Nitrogen }					
Protein .	F. III	31 ± 3	18 ± 3	3.401	< 0.01
Nitrogen }					
Hexoses .	F. I	1.357 ± 0.033	2.464 ± 0.163	2.327	< 0.05
	F. II	traces	0	—	—
Methyl-Pentoses .	F. I	0.987 ± 0.400	0.840 ± 0.276	0.318	> 0.05
	F. II	0.351 ± 0.126	0.380 ± 0.220	0.120	> 0.05
Hexosoamines .	F. I	1.173 ± 0.293	0.260 ± 0.136	11.72	< 0.01
	F. II	traces	0	—	—
Hexuronic Acids .	F. I	1.122 ± 0.170	0	—	—
	F. II	0	0	—	—
SO [—] ₄ .	Whole sample	+++	+ —	—	—

The Protein and Collagen Nitrogen of the single fractions is given as a percentage of the total Nitrogen of the samples.

All the other values are expressed as g per 100 g of proteins of the corresponding fraction.

The results of our analytical determinations, together with their statistical analysis, are reported in the Table.

The above table shows that the hexose content of the F.I. in the arteriosclerotic plaques increases in a significant way as compared with the normal aortic wall, while the methylated sugars remain unchanged. Hexosoamines and hexuronic acids are contained in the normal aortic wall in an almost equimolecular ratio; in the hyaline plaques the former decrease in a highly significant way and the latter completely disappear. The carbohydrates found in the second fraction do not assume any particular interest and are more likely due to an occasional contamination during the fractionation procedure.

The nitrogen and hydroxyproline determinations in the three aortic fractions show that there is a decrease of the normal elastic tissue of the aorta, and, at the same time, an increase of the collagenous proteins at the site of the developing hyaline plaques. The increase in collagen, together with the increase of hexoses in the arteriosclerotic aorta, could suggest the presence in the plaques of a certain amount of those polysaccharides typically bound to the collagenous and reticular fibers, as reported by SCHMITT, GROSS, and HIGHBERGER⁶ and by WINDRUM, KENT, and ESTOE⁷, which are almost exclusively composed of hexoses. This increase in hexoses, which we⁸ have observed also in the silicotic hyaline masses and in the perisplenic hyaline synechiae, seems to be a feature of the hyalinization.

The hexosoamines and the hexuronic acids, contained in the normal aorta together with the SO[—]₄ groups, confirm the presence in it of sulphated acid mucopolysaccharides, which are usually tested by histochemical methods or by the incorporation of ³⁵S in the meta-

The presence or the absence of the SO₄ groups, and their approximate amount is expressed by means of the signs: + or —.

All values are the mean ± S.E.M. of 9 determinations on different samples of normal and arteriosclerotic aortae; the 't of Student' and the value of probability are given in the last two columns.

chromatic sections of the aorta⁹. The decrease of the hexosoamines and of the SO[—]₄ groups, and the complete disappearance of the hexuronic acids in the arteriosclerotic plaques may represent a fall in the acid mucopolysaccharide quota of the aorta during arteriosclerotic disease. This is of considerable interest, since authors employing metachromatic staining reactions have reported that metachromatic substances disappear only in the initial stages of the disease, during the serous imbibition of the intima, but show again, to an even larger degree, at a later period, in the constituted arteriosclerotic lesions, either coming from the blood stream or being newly synthesized from the arterial wall.

However, a decrease of the hexosoamines contained in the arteriosclerotic aorta has also been found by KYRK and DYRBYE¹⁰, who suggest that the increased metachromasia reported during arteriosclerosis might be due to a staining alteration of the tissue mucopolysaccharides rather than to an increase of the total concentration of these compounds.

The origin of arteriosclerosis has been attributed to a depolymerization of the sulphated mucopolysaccharides, which, in the normal aorta, would maintain its elasticity and allow the filtration of the lipidic macromolecules through the arterial wall. In fact CALI¹¹ and SEIFTER, BAEDER, BECKFIELD, SHARMA, and ERICH¹², who believe that during spontaneous arteriosclerosis in man, there appear in the blood stream newly formed substances able to depolymerize the mucopolysaccharides

⁶ F. O. SCHMITT, J. GROSS, and J. H. HIGHBERGER, *Exp. Cell Research*, Suppl. 3, 326 (1955).

⁷ G. M. WINDRUM, P. W. KENT, and J. E. ESTOE, *Brit. J. exp. Pathol.* 36, 49 (1955).

⁸ B. PERNIS and E. CLERICI, *La Medicina del Lavoro* 48, 238 (1957).

⁹ A. CASTELLANI and G. GUIDOTTI, *Riv. Istochim. norm. pat.* (in press) (1957). — E. ODELBLAD and H. BOSTROM, *Acta pathol. microbiol. scand.* 31, 339 (1952). — While this paper was in preparation, a paper dealing with the isolation of mucopolysaccharides from human arterial tissue and with the determination of its chemical composition, whose results are in accord with our own, was published by M. DYRBYE and J. E. KYRK in the January issue (1957) of the *Journal of Gerontology*.

¹⁰ J. E. KYRK and M. DYRBYE, *J. Gerontology* 2, 273 (1956).

¹¹ A. CALI, *Basi teoriche e sperimentali per una patogenesi enzimatica della arteriosclerosi*. 17° Quaderno della sezione perugina della SIBS (Simonelli, Perugia 1955).

¹² J. SEIFTER, D. H. BAEDER, W. J. BECKFIELD, G. P. SHARMA, and W. E. ERICH, *Proc. Soc. exp. Biol. Med.* 83, 468 (1953).

(in spite of the presence of an antihyaluronidase factor recently found in the serum of older men by CASTELLANI and MARS¹³), they have been able to accelerate and to increase the entity of the experimental arteriosclerotic lesions of the rabbit, by using cholesterol and hyaluronidase to accelerate the depolymerization of the sulphated mucopolysaccharides.

Our results, which might suggest not only a depolymerization but even a disappearance of the sulphated mucopolysaccharides from the arteriosclerotic plaques, are therefore in accordance both with KYRK and DYRBYE¹⁰ and CALI¹¹ and SEIFTER *et al.*¹².

So far as the increased metachromasia in the arteriosclerotic aorta is concerned, while accepting the hypothesis of KIRK and DYRBYE¹², we would suggest further that the phenomenon might be due to the presence of other substances, different from the sulphated mucopolysaccharides, and particularly of the hexoses, which are significantly increased in the hyaline arteriosclerotic plaques.

B. PERNIS and E. CLERICI*

Clinica del Lavoro della Università di Milano, April 14, 1957.

Riassunto

È stata eseguita l'analisi chimica dei carboidrati, dei gruppi solforati, del collagene e dell'elastina contenuti nella parete aortica normale e nelle placche arteriosclerotiche jaline. L'analisi dei carboidrati e dei gruppi SO₄⁻ è suggestiva per la presenza di mucopolisaccaridi acidi solforati nell'aorta normale e per una loro netta diminuzione nel corso dell'arteriosclerosi. Contemporaneamente alla diminuzione di tali polisaccaridi si nota una diminuzione di elastina ed un aumento delle proteine collageni nelle placche arteriosclerotiche jaline.

¹³ A. CASTELLANI and G. MARS (personal communication).
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Haemoglobin F in *Talassemia Minor*.
Amino-Acid Composition

In a previous paper¹ evidence was given of the presence of alkali-resistant Hb, in some respects similar to Hb F, in some subjects suffering from *Talassemia Minor*. In the present communication, we report the data obtained on the amino acid composition of Hb F which we have been able to crystallize from the blood of a subject affected by this disease.

Hb obtained from erythrocytes repeatedly washed in saline was crystallized by HAUROWITZ's procedure for foetal Hb². We easily obtained hexahedral crystals typical for Hb F, well observable macroscopically, which after repeated washing in saturated ammonium sulfate appeared under the microscope to be perfectly homogeneous in shape and size.

After heat coagulation and washing off the ammonium sulfate, the precipitate was subjected to hydrolysis in 6 N HCl under reflux for 24 h. Analysis of the amino acids present in hydrolysate was performed by column chromatography on Dowex 50, X-8, according to MOORE and STEIN³, with the slight variations previously described⁴. The determinations were made in duplicate on two different 24 h hydrolysates, using for each analysis quantities of 1.61 or 2.93 mg of protein, as calculated from quadruplicate microkjeldahl and assuming a N content for Hb of 16.9%⁵.

In the Table the data obtained are reported expressed as g amino acid/100 g protein.

Comparing these data with those obtained in our previous analysis of Hb F crystallized from placental cord blood⁶, it is evident that there is a good agreement

¹ D. CAVALLINI, C. DE MARCO, A. ROSSI-FANELLI, and E. SILVESTRONI, *G. Biochim.* **3**, 307 (1954).
² F. HAUROWITZ, *Z. physiol. Chem.* **232**, 125 (1953).
³ S. MOORE and W. H. STEIN, *J. biol. Chem.* **192**, 663 (1951).
⁴ A. ROSSI-FANELLI, D. CAVALLINI, and C. DE MARCO, *Biochim. biophys. Acta* **17**, 377 (1955). – A. ROSSI-FANELLI, D. CAVALLINI, C. DE MARCO, and F. TRASARTI, *Boll. Soc. ital. Biol. sper.* **31**, 328 (1955).
⁵ W. A. SCHROEDER, L. M. KAY, and I. C. WELLS, *J. biol. Chem.* **187**, 221 (1950).
⁶ A. ROSSI-FANELLI, D. CAVALLINI, C. DE MARCO, and F. TRASARTI, *Boll. Soc. ital. Biol. sper.* **31**, 328 (1955).

The amino acid composition of 24 h hydrolysate of the alkali-resistant fraction in *Talassemia Minor*.

	Talassemia Minor				Mean	Hb F ⁶	Cooley's anemia ⁷
Aspartic	9.93	9.63	10.32	9.93	9.95	9.59	10.90
Theronine	6.94	7.10	5.82	6.60	6.61	6.98	6.30
Serine	6.69	6.94	5.91	6.28	6.45	6.39	5.50
Glutamic	7.81	8.20	6.96	7.29	7.56	6.81	7.85
Proline	3.01	4.32	5.49	—	4.27	4.70	4.05
Glycine	4.85	4.21	4.42	3.58	4.26	3.98	4.30
Alanine	9.02	10.57	8.04	7.92	8.88	8.52	9.65
Valine	11.01	10.63	8.20	8.67	9.62	8.34	9.35
Methionine	2.28	2.31	1.69	1.67	1.98	1.84	—
Isoleucine	1.37	2.28	1.48	1.32	1.61	1.49	1.75
Leucine	15.08	16.52	15.30	13.06	14.99	13.67	15.10
Tyrosine	3.13	2.63	2.88	—	2.88	2.68	3.15
Phenylalanine	9.93	8.82	6.99	—	8.58	8.83	7.90
Lysine	9.33	9.99	9.38	—	9.56	12.08	9.70
Histidine	7.58	7.61	7.25	—	7.48	7.04	7.25
Arginine	2.91	3.11	2.83	2.79	2.91	3.11	3.30
					107.59		

between these values, the small differences being imputable to the standard error of the method. The only appreciable difference is in lysine content, and this can be ascribed to its relative instability in acid solutions⁶.

Recently HUISMAN *et al.*⁷ have analyzed the amino acid composition of alkali resistant Hb separated by chromatography on Amberlite IRC-50 from blood haemolysate of a patient suffering from Cooley's anemia (*Talassemia Major*). Our data are also fully comparable with those obtained by these authors, and reported in the Table for comparison. HUISMAN *et al.* come to the conclusion that Hb F and the alkali resistant fraction of Cooley's anemia are in all probability the same protein.

Our results on crystallization and amino acid composition further support the hypothesis that alkali-resistant Hb present in *Talassemia Minor* and in Cooley's anemia is identical to Hb F.

C. DE MARCO and FRANCESCA TRASARTI

Institute of Biological Chemistry, University of Rome (Italy), June 3, 1957.

Riassunto

Dal sangue di un soggetto affetto da *Talassemia Minor* si sono ottenuti cristalli tipici di Hb F.

L'analisi della composizione in aminoacidi di tali cristalli porta a concludere che la frazione di Hb alcali-resistente presente in questa malattia e l'Hb F sono identiche.

⁷ T. H. J. HUISMAN, H. K. PRINS, and P. C. VAN DER SCHAAF, *Exper.* 12, 107 (1956).

A Female-sterile Mutant
(Deep Orange) of *Drosophila melanogaster*
Increasing Isoxanthopterine Content

Isoxanthopterine has been found and identified in the tissues of both normal and mutant *Drosophila melanogaster*¹. In the mutants *white* and *brown*, it disappears from the tissues during the first few days of imaginal life and it is never present at any stage of development in the mutant *rosy*³. In the more than 25 eye colour mutants which have been examined⁴, none has increased the amount of isoxanthopterine present in the tissues.

The sex-linked recessive female-sterility factor *deep orange* (*dor*) has an effect on eye colour⁵. Eyes of both males and females are light orange in newly emerged flies and darken to a deeper orange with age. A comparison of the isoxanthopterine content of wild type (Sevelen line) and *dor* flies at four different developmental stages shows a clear difference between mutant and wild type flies in isoxanthopterine content (Tables I and II). In *dor* females, the amount of isoxanthopterine in-

¹ E. HADORN and H. K. MITCHELL, *Proc. nat. Acad. Sci., Wash.* 37, 650 (1951). – S. NAWA and T. TAIRA, *Proc. imp. Acad. Japan* 30, 632 (1954). – E. HADORN, *Exper.* 10, 483 (1954). – H. S. FORREST and H. K. MITCHELL, *J. Amer. chem. Soc.* 77, 4865 (1955). – M. VISCONTINI, E. LOESER, P. KARRER, and E. HADORN, *Helv. chim. Acta* 38, 2034 (1955).
² E. HADORN, *Exper.* 10, 483 (1954).
³ E. HADORN and I. SCHWINCK, *Nature* 177, 940 (1956); *Z. Vererbungslehre* 87, 528 (1956).
⁴ E. HADORN and H. K. MITCHELL, *Proc. nat. Acad. Sci., Wash.* 37, 650 (1951). – E. HADORN, *Exper.* 10, 483 (1954). – E. HADORN and I. SCHWINCK, *Nature* 177, 940 (1956); *Z. Vererbungslehre* 87, 528 (1956).
⁵ D. J. MERRELL, *Amer. Naturalist* 81, 399 (1947).

Table I

Differences in isoxanthopterine content of + and *dor* males at different developmental stages, expressed in percentage of + value.

Developmental stage	+ ♂♂*		<i>dor</i> ♂♂			<i>t</i>	<i>p</i>
	<i>n</i>	SE	<i>n</i>	$\bar{x}$	SE		
Prepupa	26	1.41	28	63.82	2.26	12.54	< 0.001
50 h pupa	21	1.62	21	72.57	2.11	10.63	< 0.001
Emergence (bodies only)	15	1.82	16	99.31	2.21	0.19	not significant
4 day imago (bodies only)	13	1.86	14	74.50	3.59	4.01	< 0.001

* Because of variations from chromatogram to chromatogram it was not always possible to make a direct comparison of fluorescent values; therefore the mean value of the + readings on any one sheet was given a value of 100.0% and all readings converted to equivalent percentage values. The figures in the third column give the standard error of + male readings.

Table II

Differences in isoxanthopterine content of +, *dor* and *CIB/dor* females at different developmental stages, expressed in percentage of + value.

Developmental Stage	+ ♀♀*		<i>dor</i> ♀♀			<i>t</i>	<i>p</i>	<i>CIB/dor</i> ♀♀			<i>t</i>	<i>p</i>
	<i>n</i>	SE	<i>n</i>	$\bar{x}$	SE			<i>n</i>	$\bar{x}$	SE		
Prepupa	8	2.99	17	117.65**	3.41	4.05	0.001					
50 h pupa	12	2.63	11	165.28	9.51	6.73	0.001	11	143.91	3.30	10.48	< 0.001
Emergence (bodies only)	16	3.54	14	207.0	14.21	7.31	0.001	15	175.10	5.21	12.25	< 0.001
4 day imago (bodies only)	25	2.40	23	233.43	6.78	18.38	0.001	19	131.74	4.40	6.30	< 0.001

* As in Table I, all figures are converted to percentage of + mean value on each chromatogram, the + value being equal to 100.0%.
** At this stage, it is not possible to distinguish between *dor* and *CIB/dor* females; this measurement represents their combined values.

creases during development until more than twice the normal content is present. Females heterozygous for *dor* also show increased amounts of isoxanthopteryne in all developmental stages studied. In mutant males, on the other hand, the isoxanthopteryne level remains below that of normal males during pupal development, and only at hatching is the amount equal to that in + males.

Pigmentation of *dor* testes occurs precociously, and the testes of newly emerged mutant males are often yellow, while those of newly emerged + males are colourless or only faintly tinged with yellow. By 4 days, however, the normal testes are slightly darker than those of mutant males. Differences in content of fluorescent substances in the bodies of + and *dor* males are probably

Table III
Differences in the pterine content of the heads of 4 day + and +/*dor* adult females expressed in percentage of + value.

Pterines (Heads only)	+ ♀♀		+/ <i>dor</i> ♀♀			<i>t</i>	<i>p</i>
	<i>n</i>	SE	<i>n</i>	$\bar{x}$	SE		
Red	17	2.69	20	121.80	3.56	4.92	< 0.001
'Xanthopteryne'	19	2.96	25	151.88	5.65	8.10	< 0.001
'Sepia' pterine	12	4.88	14	114.64	2.80	2.60	< 0.05
HB ₁ + HB ₂	12	4.67	14	148.43	7.70	5.30	< 0.001

4 days after hatching, the amount in *dor* males has declined to three-fourths that of the + males.

related in part to differences in the rate and amount of testes pigmentation.

Other fluorescent substances are also affected in the mutant flies. In the bodies of four-day-old male *dor* flies, the green fluorescent substance (tentatively identified in our laboratory as xanthopteryne by Dr. ZIEGLER-GÜNDER, unpublished) is reduced to $59.8 \pm 4.2\%$ of the amount of + males of the same age. 'Xanthopteryne' measurements were not made of female bodies. At least 3 components of the fluorescent spots designated as 4 and 5 by HADORN and MITCHELL⁶ have been identified: the 'sepia' pterine (yellow fluorescence)⁷ and two blue fluorescent substances, HB₁ (2-amino-6-hydroxy-pterine)⁸ and HB₂⁹. All of these substances are present in amounts about 3 times that of the normal males in newly emerged *dor* males; by 4 days, however, there are no significant differences between normal and mutant males in these substances in the body. The bodies of newly emerged and four-day-old *dor* females contain 3 times as much of the fluorescent substances of the 4/5 spots as do those of + females of the same age.

Malpighian tubules of *dor* flies of both sexes are deep yellow in colour¹⁰ and are thicker and stiffer than normal Malpighian tubules. This is due to the accumulation of large amounts of excretory material in the lumen (*cf. rosy*³). The tubules of four-day-old *dor* flies contain 3 to 6 times more of the fluorescent substances than those of + flies of the same age. The blue components of 4/5, which are present only as traces in the tubules of + flies, are present in considerable concentration in *dor* tubules.

Heads of newly emerged and four-day-old flies were measured separately. Red pigments and 'xanthopteryne' are reduced in both male and female *dor* to about 10% of the normal amounts. The 'sepia' pterine is reduced to about half the amount found in + flies, but there is no significant difference between + and *dor* flies in the blue components 4/5. Red pigments, 'xanthopteryne' and the components of 4/5 are all increased in the heads of +/*dor* hybrids (Table III). Measurements were made of +/*dor* because of the reduction in the size of the eye in *CIB/dor* flies due to the *Bar* gene; measurements of the pterines in the bodies of +/*dor* flies gave results comparable to those obtained from *CIB/dor* flies of the same age. Until the biochemical interrelations of the various pterines are better understood, it is not possible to give an adequate explanation of the results obtained in the heterozygous female.

The different biochemical patterns exhibited by *dor* males and females are of interest in relation to the fact that lethal embryos from homozygous *dor* females exhibit two characteristic phenocritical periods which are dependent upon the sex of the embryo¹⁰. Preliminary results indicate that amino acid metabolism is also affected to a different degree in the two sexes.

All measurements were made on one-dimensional chromatograms developed in propanol-amonia. Whole prepupae and 50 h pupae, and heads and bodies of newly emerged and four-day-old flies, were squashed directly on the filter paper. Measurements of the fluorescent intensity of various spots were made by the direct method first used by HADORN and KÜHN¹¹.

It is a pleasure to acknowledge the help and warm hospitality of Professor E. HADORN of the Zoologisch-vergl. Anatomisches Institut of the University of Zürich. Dr. H. BURLA and Dr. I. ZIEGLER-GÜNDER have also given much help and advice. The work was carried out during the tenure of a post-doctoral fellowship from the National Science Foundation, Washington, D.C.

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Zoologisch-vergleichendes Anatomisches Institut, University of Zürich, May 8, 1957.

Zusammenfassung

Der Bestand an fluoreszierenden Stoffen in verschiedenen Entwicklungsstadien der Weibchen-steril-Mutante

⁶ E. HADORN and H. K. MITCHELL, Proc. nat. Acad. Sci. Wash. 37, 650 (1951).
⁷ H. S. FORREST and H. K. MITCHELL, J. Amer. chem. Soc. 76, 5656, 5658 (1954).
⁸ M. VISCONTINI, E. LOESER, P. KARRER, and E. HADORN, Helv. chim. Acta 38, 1222 (1955). - H. S. FORREST and H. K. MITCHELL, J. Amer. chem. Soc. 77, 4865 (1955).
⁹ H. S. FORREST and H. K. MITCHELL, J. Amer. chem. Soc. 77, 4865 (1955).

¹⁰ S. J. COUNCE, Z. Vererbungslehre 87, 443 (1956).
¹¹ E. HADORN and A. KÜHN, Z. Naturf. 8b, 582 (1953).
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deep orange wurde papierchromatographisch festgestellt. In allen geprüften Stadien von *dor/dor*- und *ClB/dor*-Weibchen ist mehr Isoxanthopterin nachweisbar als bei +-Weibchen. Bei Männchen der Mutante findet sich in Puppenstadien weniger Isoxanthopterin als bei +-Männchen; nur bei frisch geschlüpften Fliegen entsprechen sich normale und *dor*-Männchen in ihrem Isoxanthopterin-Gehalt. Bei *dor/dor*- und *dor/Y*-Tieren sind ausserdem noch andere Pterine betroffen. So zeigen die Köpfe der *dor*-Mutante weniger rote Pigmente, sowie mehr «Xanthopterin» und «Sepia-Pterin» als Fliegen des Wildtyps, dagegen kommen HB₁ und HB₂ in normaler Menge vor. Im Kopf der heterozygoten Weibchen werden für alle diese Pterine Werte gemessen, die sogar den Wildtypus beträchtlich übertreffen. Bei *dor/dor* und *dor/Y* findet sich in den Malpighischen Gefässen eine stark erhöhte Konzentration an fluoreszierenden Stoffen.

Taxonomical Implications of the Properties of a Riboflavin-producing Mutant Yeast

Introduction.—SUBRAMANIAM and RANGANATHAN¹ isolated a top yeast, BY 2, after treatment of a brewery bottom yeast with acenaphthene for 90 days. Studies on the biochemical properties of the mutant revealed² that the strain is capable of synthesizing and excreting into the medium a considerable amount of riboflavin under simple cultural conditions. The remarkable stability of this property of the mutant has been varified through continued observation and study³ during the past 10 years.

Certain yeasts⁴ and yeast-like organisms⁵ are known to be endowed with this property of producing large amounts of riboflavin. The question was posed whether the artificial alteration of the chromosomal constitution¹ of the parent strain had resulted in the creation of one of these organisms having acknowledged property of riboflavin production. An alternative hypothesis would be that the mutant yeast, BY 2, is a new species of yeast created by an induction of mutation. In order to elucidate this point, the properties of the mutant yeast were analysed with regard to the major characteristics having taxonomical implications.

Observations.—*I. Cultural characteristics.* Typical giant colony of the mutant in barley malt agar is rough and has a wavy outline with a distinct ring around the periphery. The young colonies are white and dry; they become moist, slimy and brown with age.

In *still* liquid culture, the strain forms distinct pellicles which are white, dull and wavy in character. They appear as islets and subsequently unite to form a distinct surface film; the rest of the medium remains clear. On prolonged incubation, the film gains in thick-

ness and finally settles down forming a flocculant sediment and leaves a distinct ring around the surface. A homogeneous growth is obtained in *agitated* liquid media. Deep yellow colour due to the excretion of riboflavin is evident in appropriate liquid media on prolonged incubation.

II. Morphological characters.—The cells of the mutant show a wide degree of polymorphism. The cell shape varies from bulb-shape through long oval to fairly elongated ones. Polymorphism is less evident in young culture in agitated liquid media where the range of size of the cells is usually $(5-9) \mu \times (2.5-3.5) \mu$. The reproduction of the cells is by multilateral budding and the cells have a very distinct tendency to become pseudomycelial. Sporulation is absent.

III. Physiological characteristics

(1) *Sugar fermentation.*—Fermentation tests in Eimhorn tubes were carried out with media composed of 1% peptone and 4% of the individual sugars, *viz.*, glucose, galactose, maltose, sucrose and lactose. No gas formation was observed in any of the tubes even on prolonged incubation for 15 days. The mutant yeast is a purely non-fermentative organism.

(2) *Sugar utilization.*—Experiments on the assimilation of sugar in Laurent's medium enriched with the vitamin supplement solution show that the mutant yeast can utilize glucose, galactose, maltose and sucrose. No growth is observed in medium containing lactose as the sole source of carbon.

(3) *Assimilation of nitrogen.*—Lodder's medium with the vitamin supplements was used for these tests. Nitrogen was supplied at a level of 21 mg/ml in the form of peptone, urea, asparagine hydrate, ammonium sulphate and potassium nitrate in the individual media tested. The results of these experiments show that very good growth of the mutant is obtained in medium containing peptone as the sole source of nitrogen. Ammonium sulphate, asparagine hydrate and urea can also serve independently as suitable sources of nitrogen for the growth of the mutant. The mutant is incapable of utilizing potassium nitrate as a source of nitrogen.

(4) *Utilization of ethanol.*—A simple inorganic medium containing MgSO₄, 7H₂O; KH₂PO₄; (NH₄)₂SO₄ and enriched with biotin was used for these tests. A positive growth is obtained when ethanol is incorporated at a level of 2% by volume in this medium. The mutant is capable of utilizing ethanol as the sole source of carbon in the medium.

(5) *Effect on litmus milk.*—Tubes containing sterile litmus milk with and without calcium lactate were inoculated with the mutant yeast and incubated at 30°C. Results of observation after 3 weeks of incubation show that: a) the colour of litmus milk changes to blue, and b) in tubes containing added calcium lactate, there is coagulation along with the changing of the colour to blue.

Discussion. An analysis of the properties of the mutant yeast, BY 2, detailed above shows that the strain resembles in a general way *Candida scotii* DIDDENS et LODDER⁶ but differs from it in the following characters:

⁶ J. LODDER and N. J. W. KREGER-VAN RIJ, *The yeasts — a taxonomic study* (Amsterdam 1952).

¹ M. K. SUBRAMANIAM and B. RANGANATHAN, *Nature* 157, 49 (1946).

² K. K. MITRA, *J. sci. industr. Res.* 8B, 236 (1949); 11B, 109 (1952).

³ K. V. GIRI and P. R. KRISHNASWAMI, *J. Bact.* 67, 309 (1954). — K. K. MITRA, *J. sci. industr. Res.* 14C, 21 (1955); 15C, 257 (1956). — K. V. GIRI and P. R. KRISHNASWAMI, *J. sci. industr. Res.* 13A, 106 (1954).

⁴ P. R. BURKHOLDER, *Arch. Biochem.* 3, 121 (1943).

⁵ F. W. TANNER jr. and J. M. VAN LANEN, *J. Bact.* 54, 38 (1947). — A. GUILLIERMOND *et al.*, *C. r. Acad. Sci.* 201, 1077 (1935).

Properties	<i>C. scotii</i>	BY 2
1. Growth in malt extract .	Sediment	Pellicle
2. Assimilation of potassium nitrate.	Positive	Negative
3. Utilization of ethanol . .	Occasionally some growth.	Positive
4. Riboflavin excretion . .	Nil	Positive

The question arises as to how far these differences in properties are of taxonomic importance. Pellicle formation has been used as the criterion for differentiating certain species of *Candida* (e.g. *C. mycoderma* from *C. zeylanoides*)⁶ capable of assimilating only glucose. Similarly, the ability to utilize potassium nitrate separates *C. pelliculosa* from *C. albicans* and *C. parapsilosis*. Finally, while the mutant yeast, BY 2, excretes into the medium an appreciable quantity of riboflavin, there is no record of a similar behaviour in *C. scotii*.

Therefore the riboflavin producing mutant yeast, BY 2, has to be considered as a new species, and the name⁷ *Candida ghoshii* n. sp. is proposed for it.

Its position in the key to the species of the genus *Candida* given by LODDER *et al.*⁶ is indicated below: No sugar fermentation. Glucose, galactose, saccharose and maltose assimilated.

- a) Early pellicle formation; nitrate not assimilated; riboflavin excreted ... *C. ghoshii* n. sp.
- b) No pellicle; nitrate assimilated; no riboflavin excretion ... *C. scotii*.

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K. K. MITRA

Jadavpur University, Calcutta (India), May 6, 1957.

Résumé

Pour une détermination taxonomique, nous avons étudié les caractères morphologiques, physiologiques et culturels d'une levure mutante produisant la lactoflavine. Tout d'abord cette souche mutante est produite en traitant à l'acenaphthène et levure de bière à fermentation basse. Cette souche est asporogène et présente des formes pseudomycéliques. Elle est incapable de fermenter le glucose et n'utilise pas le KNO₃ comme source d'azote. Elle n'assimile pas le lactose. Après une étude approfondie sur les différents caractères de cette souche, nous estimons qu'elle peut être considérée comme une nouvelle espèce de levure du genre *Candida*. Nous l'avons appelée *C. ghoshii* n. sp.

⁷ The species is named after Sir J. C. GHOSH, who gave a fillip to these investigations on yeast at the Indian Institute of Science, Bangalore.

Glycolitic Enzymes in the Human Amniotic Fluid

Very few enzymatic activities have so far been described in the amniotic fluid. Only scanty data exist on cholinesterase¹, phosphatase², and lysozima³.

As it is well known that glucose, fructose and, probably, other reducing sugars are present in the amniotic fluid⁴, it was a matter of interest to investigate whether glycolytic activities could be detected in this liquid.

The following enzymatic activities have been examined: phosphohexose-isomerase, ribose-5-phosphate-isomerase, aldolase and lactic-dehydrogenase.

Determinations have been made of the total protein content of the amniotic fluids under examination.

Experimental.—The samples of amniotic fluid (of about 15 ml each) were collected from pregnant women at term by transabdominal puncture, *intrapartum* by vaginal puncture of amniotic sac or during Caesarean section.

The samples were filtered and stored at 0° for no longer than 24–48 h. Samples contaminated by blood or meconium were discarded.

The protein content was determined by the Goa's micromethod⁵.

Phosphohexoso-isomerase.—The activity of this enzyme was determined according to BODANSKY⁶: in a centrifuge tube 0.3 ml veronal buffer pH 7.8 0.1 M, 0.3 ml glucose-6-phosphate sodium salt 0.045 M, 0.2 ml amniotic fluid were pipetted. The mixture was incubated at 37° for 1 h and the reaction stopped by addition of 3 ml of 20% trichloroacetic acid (TCA). After centrifugation, fructose was determined on 2 ml of the supernatant according to ROE⁷.

Aldolase.—Determinations were carried out in a centrifuge tube mixing 1 ml of TRIS-HCl buffer pH 7.4 0.1 M, 0.25 ml hydrazine 0.56 M pH 7.4, 0.25 ml iodoacetate 0.002 M, 0.25 ml distilled water and 1 ml of amniotic fluid and 0.25 ml hexose 1-6-diphosphate Na salt 0.06 M⁸.

The mixture was incubated for 1 h at 37°, and successively deproteinized by 3 ml of 10% TCA. After centrifugation, triosephosphate was determined on 1 ml of the supernatant according to SIBLEY and LEHNINGER⁹. For standardization of method, in some samples alkali-labile phosphorus was determined¹⁰ in duplicate.

Ribose-5-phosphate-isomerase.—This activity was tested according to AXELROD¹¹: 0.5 ml of TRIS-HCl buffer pH 7.4 0.1 M, containing 0.5 mg Ribose-5-phosphate (Ba salt) were mixed with 0.5 ml of amniotic fluid; after incubation at 37° for 30 min ribulose-5-phosphate

¹ R. PICCOLI and G. LONGO, Boll. Soc. ital. Biol. sper. 23, 941 (1947).
² F. SEELICH and H. EHRLICH-GOMOLKA, Enzymologia 15, 96 (1951).
³ G. VECCHIETTI, Quad. Clin. Obstetr. Gin. 3, 233 (1948).
⁴ H. MOHS, Arch. Gynäkol. 147, 532 (1931). — A. CANTAROW, H. STUCKERT, and R. C. DAVIS, Surg. Gyn. Obstetr. 57, 23 (1933). — ICHIJO MASAYOSHI, Japan J. Med. Sci. II Biochem. 2, 359 (1934). — C. MASUKO, Japan J. Obstetr. Gyn. 23, 102 (1940). — P. MAGNIN, E. ZAHEDI, and G. PROST, Gyn. Obstetr. 51, 375 (1952).
⁵ J. GOA, J. Clin. Lab. Invest. 5, 218 (1953).
⁶ O. BODANSKY, J. biol. Chem. 202, 840 (1953).
⁷ J. H. ROE, J. biol. Chem. 107, 15 (1934).
⁸ F. BRUNS, Biochem. Z. 325, 156 (1954).
⁹ J. A. SIBLEY and J. LEHNINGER, Nat. Cancer Inst. (Bethesda) 9, 303 (1949).
¹⁰ I. BERENBLUM and E. CHAIN, Biochem. J. 32, 589 (1956).
¹¹ B. AXELROD and R. YANG, J. biol. Chem. 209, 867 (1954).

Protein content and glycolytic activities of human amniotic fluid

Samples	Protein ¹	Aldolase ²	PEI ³	LDI ⁴	PRY ⁵
1	208	0.278	0.340	0.250	—
2	296	0.094	0.108	0.054	—
3	206	0.237	0.340	0.155	—
4	140	0.121	—	—	—
5	262	0.244	0.150	0.056	—
6	193	0.057	0.052	0.088	0.045
7	164	0.025	0.045	0.079	0.095
8	148	0.048	0.018	0.034	—
9	255	0.040	0.097	0.029	0.149
10	241	0.149	0.059	0.021	—
11	302	0.087	0.108	0.043	0.023
12	182	0.312	0.185	—	—
13	145	0.067	0.140	0.137	0.034
14	200	0.055	0.106	0.067	0.318
15	160	0.056	0.090	—	0.220
16	206	—	0.106	—	0.089
17	205	0.024	—	—	0.263
18	162	0.019	—	—	0.405
19	175	0.103	—	—	0.240
20	217	0.032	—	—	0.350
21	207	0.034	—	—	0.130
Mean values	203 ± 46	0.104 ± 0.091	0.149 ± 0.101	0.181 ± 0.126	0.085 ± 0.066

¹ Protein = mg%/ml.² Aldolase = μ M hexosediphosphate/h/mg of protein.³ PEI = phosphohexoso-isomerase = mg fructose/h/mg of protein.⁴ LDI = lactic-dehydrogenase = μ M/min/mg of protein.⁵ PRY = phosphoriboso-isomerase = μ M ribulose/h/mg of protein.

was determined with the sulfuric acid-cistein-carbazole reagent.

Lactic dehydrogenase.—This activity was determined spectrophotometrically¹² as follows: in a cuvette of the Beckman spectrophotometer were pipetted: 0.4 μ M DPNH in 0.1 ml, 3 μ M piruvate in 0.1 ml, 0.3 ml NaHCO₃ 0.02 M, 2.5 ml phosphate buffer 0.1 M pH 7.8.

The reaction was started by addition of 0.1 ml amniotic fluid and the decrease of O.D. at 340 m μ was followed for 10 min at room temperature taking readings every minute.

In the Table the data obtained in these experiments are reported with their mean and standard deviation. It is evident that all enzymatic activities tested are present in considerable amounts in the amniotic fluid.

The enzymatic activity referred to the protein content is much higher in amniotic fluid than in blood serum of either mother or newborn¹³.

The values obtained from the different samples are considerably scattered. It has not been possible to relate such differences to the way in which the fluid was obtained nor the time the liquid have been stored.

The protein values obtained are in good agreement with those previously reported by other authors¹⁴.

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Institute of Biological Chemistry, University of Rome (Italy), April 1, 1957.

¹² F. KUBOWITZ and P. OTT, *Biochem. Z.* **314**, 94 (1943). — B. R. HILL and C. LEVI, *Cancer Res.* **14**, 513 (1954).

¹³ E. ANTONINI, C. DE MARCO, and S. MARI, *Boll. Soc. ital. Biol. sper.* **32**, 589 (1956). — C. DE MARCO, E. ANTONINI, and S. MARI, *Arch. Sci. Biol.* **41**, 286 (1957). — P. FIORETTI (personal communication).

¹⁴ F. A. URANGA IMAZ and A. GASCON, *Obstetr. y Ginec. latino-amer.* **8**, 237 (1950). — F. HANON, M. COQUOIN-CARNOT, and P. PIGNARD, *Le liquide amniotique* (Ed. Masson 1954), p. 127.

Riassunto

È stata messa in evidenza nel liquido amniotico di donne a termine di gravidanza la presenza di alcuni enzimi glicolitici. Sono riportati i dati quantitativi ottenuti per l'attività della fosforiboso-isomerasi, aldolasi, fosfoesoso-isomerasi e lattico-deidrogenasi. È stato determinato inoltre il contenuto proteico dei campioni di liquido amniotico presi in esame.

Post Nephrectomy Increase in Serum Ribonuclease Activity after Total Hepatectomy or Nitrogen Mustard Derivatives Administration*

It has been previously shown that serum ribonuclease (Rase) activity markedly increases after bilateral nephrectomy in the rat¹, this phenomenon being observed in animals undergoing a number of experimental procedures, including 'functional evisceration'². Although our results suggested that the liver is not the source of the enzyme increase, the disadvantages of leaving that organ *in situ*³ led us to study the effects of total hepatectomy and evisceration. These were performed by the two stage technique of INGLE⁴ on Wistar rats of both sexes (average body weight 250 g). The second stage was carried out under ether anesthesia, and was immediately followed by bilateral nephrectomy, non-nephrectomised

* Aided by grants from 'Conselho Nacional de Pesquisas' and The Rockefeller Foundation.

¹ M. RABINOVITCH and S. R. DOHI, *Amer. J. Physiol.* **187**, 525 (1956).

² J. A. RUSSEL, *Amer. J. Physiol.* **136**, 95 (1942).

³ F. C. MANN, *Medicine* **6**, 419 (1927).

⁴ D. J. INGLE, *Exp. med. Surg.* **7**, 34 (1949).

animals serving as controls. The rats were kept at 27°C after operation, there being an average rectal temperature drop of 4°C. Blood was obtained from the jugular veins at nephrectomy and 4 h later.

The presence of Rase in leucocytes⁵, and the increase in urine Rase activity in myeloid leukemia⁶, prompted us to investigate whether in granulopenic animals the same effect of bilateral nephrectomy would be obtained. For that purpose, rats of both sexes, 200–250 g body weight, received either 2 mg/kg of methyl-bis-β-chloroethylamine hydrochloride⁷ ('Dichloren' Ciba) intravenously, or two 20 mg/kg doses of 1–4 dimethanesulfonylbutane⁸ ('Myleran' Burroughs Wellcome) intraperitoneally 5 days apart. The 'Dichloren' treated rats were nephrectomised on the 5th day, and the rats treated with 'Myleran' on the 12th day after the initial administration of the drugs. The animals were bled at the start of the experiments, at the time of nephrectomy and 4 h later. Comparison of samples of the first two stages showed that both drugs had no effect on serum Rase activity. Total and differential white blood cell counts were obtained from these animals as well as from the controls.

Results are to be found in the Tables I and II.

It can be seen that in the eviscerated-totally hepatectomized animals, and in the animals treated with the nitrogen mustard derivatives, the increase in serum Rase activity after nephrectomy is not significantly different from that of control animals. The pronounced

lymphoid aplasia observed in the 'Dichloren' treated rats gives support to our assertion¹ that lymphoid tissue is presumably not involved in the post-nephrectomy increase in serum Rase activity.

The above data, together with those reported previously, tend to rule out, therefore, either the liver, pancreas, digestive tract, adrenals or hemopoietic tissues, as the sole sources of the enzyme increase. Nevertheless, we cannot exclude the possibility that these, together with other tissues and organs, release the enzyme into the blood, so that their individual contribution would be undetectable under the present conditions, for each of them would then represent but a small fraction of the total mass of the body.

Acknowledgments.—We thank 'Produtos Químicos CIBA, S. A.' for the 'Dichloren' and Dr. M. A. JAMRA for the 'Myleran' used.

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Laboratory for Cell Physiology, Faculty of Medicine, University of São Paulo (Brazil), April 23, 1957.

Résumé

L'augmentation de l'activité de la ribonucléase du sérum, qui fait suite à la néphrectomie bilatérale chez le rat n'est pas modifiée par une éviscération préalable avec hépatectomie totale ou administration de moutardes azotées. Ces résultats indiquent que ni le foie ni les leucocytes et organes hémapoïétiques ne peuvent être considérés comme sources exclusives de cette augmentation.

Table I

Serum Rase activity in hepatectomised-eviscerated rats with and without bilateral nephrectomy

Treatment	Number of animals	Serum ribonuclease activity*
Hepatectomised + evisceration . . .	5	97.9 ± 18.6
Hepatectomised + eviscerated + bilat. nephrectomy	8	161.0 ± 10.7

* Mean in % of pre-nephrectomy activity ± S.E.

Table II

Total leucocyte counts, total neutrophil counts and serum Rase activity in rats nephrectomised after N mustard derivatives treatment

Treatment	No. of animals	Total leucocyte counts ¹	Total granulocyte counts ¹	Serum Rase activity ²
Controls . .	8	125.0 ± 15.3 ³	127.3 ± 16.2	229.9 ± 17.7
'Dichloren' . .	5	6.4 ± 2.5	7.9 ± 3.7	274.4 ± 16.8
'Myleran' . .	4	27.0 ± 3.5	13.9 ± 4.9	204.2 ± 23.8

¹ Counts taken immediately before nephrectomy in % of initial counts before drug administration.
² In % of pre-nephrectomy activity.
³ Mean ± S.E.

⁵ R. J. DUBOS and C. M. McLEOD, *J. exp. Med.* **67**, 793 (1938).
⁶ J. ALEXANDROVICZ and J. SPIRER, *Le Sang* **26**, 212 (1955).
⁷ I. GRAEF, D. A. KARNOFSKY, V. B. JAGER, B. KRICHEWSKY, and H. W. SMITH, *Amer. J. Pathol.* **24**, 1 (1948).
⁸ A. HADDOW and G. M. TIMMIS, *Lancet* **1**, 207 (1953).

Antibodies Against Connective Tissue in Collagen Diseases

Autoimmunizing processes are reported as possible factors in the pathogenesis of acute rheumatism and recently in other diseases included among collagen diseases¹. It is natural that attention was also directed to the ground substance. A component of the ground substance—the hyaluronic acid—in the tests of some authors proved to be nonantigenic². As noted by SEIFTER³, TINACCI and BENASSI recently ascertained an antigenic response in rats and rabbits after a single dose of purified hyaluronate prepared from human umbilical cords. An attempt was made to ascertain whether auto-antibodies, reacting with isolated components of the ground substance, develop in collagen diseases. From the series of constituents of ground substance, attention was first paid to hyaluronic acid. Since the hyaluronic acid-hyaluronidase system is strikingly disorganized in febris rheumatica, the question was first investigated in this disease.

Material and Methods.—The sera of the rheumatic patients and controls were stored at – 25°C and elaborated within 72 h of collecting the blood. Hyaluronic acid (HA) was prepared in the form of potassium hyalur-

¹ P. A. CAVELTI, *J. Allergy* **26**, 95 (1955). – V. WAGNER, *Vnitřní lékařství* **1**, 481 (1955). – V. WAGNER and V. REJHOLEC, *Ann. Rheum. Dis.* **15**, 364 (1956).
² K. MEYER, *Physiol. Rev.* **27**, 335 (1947). – H. F. SWIFT, *Amer. J. Med.* **2**, 168 (1947). – J. SEIFTER, D. H. BAEDER, and W. J. BEDKFIELD, *Proc. Soc. exp. Biol. Med.* **85**, 444 (1954).
³ J. SEIFTER, D. H. BAEDER, and W. J. BEDKFIELD, *Proc. Soc. exp. Biol. Med.* **85**, 444 (1954).

onate by the method of McCLEAN⁴ in the modification of HARRIS *et al.*⁵.

Antibodies against hyaluronic acid were demonstrated either by precipitation in capillary tubes or by haemagglutination with unprepared erythrocytes or by collodion agglutination. The results obtained by collodion agglutination only are given since this particular method proved to be the most suitable. The collodion agglutination was performed by the method of CAVELTI⁶ in the modification of WAGNER⁷. For the collodion agglutination the hyaluronic acid was dissolved in physiological saline in the ratio of 5 mg to 1 ml of the solvent under sterile conditions at 37°C. All the tests were performed with one batch of HA. The reaction was read off by tapping the bottom of the test-tube while observing it with side illumination. Positive reactions are characterized by forming floccules which do not break up on shaking, and negative reactions by diffuse turbidity. The test was appraised only if all the controls (antigen with collodion, serum with collodion, serum of a healthy person with antigen and collodion) were completely negative.

nective tissue and skin only. All values of χ^2 are highly significant. By selecting the material into groups according to the titre 0, 4–16, 32 and more, the same significance was ascertained as in the collected material. For the other antigens employed (bone-marrow, liver, spleen, brain) the values of χ^2 are not significant on 5% level and thus the hypothesis of independence cannot be rejected.

Discussion.—By means of collodion agglutination, autoantibodies against one of the components of the ground substance—the hyaluronic acid—were successfully demonstrated in the serum of patients with febris rheumatica. A comparison of the serum reaction with HA and with other nonpurified tissue antigens showed a dependence directly proportional to the content of HA in the tissue concerned: the highest dependence found, between the skin antigen and HA, seems to be conditioned by the high concentration of HA in the skin unlike the connective tissue and myocardium where its content is essentially lower and unlike further organs where it is found in insignificant concentrations⁸. The negative results with HA with the simultaneous positive finding

Occurrence of Autoantibodies against Hyaluronic Acid and Myocardium, Connective Tissue and Skin in Rheumatic Fever.

Hyaluronic Acid	Myocardium			Connective Tissue			Skin		
	—	+	Total	—	+	Total	—	+	Total
—	91 (72.3)	16 (34.7)	107	92 (64.6)	12 (39.3)	104	92 (54.7)	20 (57.3)	112
+	90 (108.7)	71 (52.3)	161	69 (96.3)	86 (58.6)	155	30 (67.3)	108 (70.7)	138
Total	181	87	268	161	98	259	122	128	250

Results.—The sera investigated were divided into two groups. The first group consisted of 127 healthy subjects for the most part blood-donors. None of the control sera gave a positive result in collodion agglutination with HA. In the second group 273 sera from 115 patients hospitalized with a still active form of febris rheumatica were examined. The latter group includes cases with different stages of the disease and with varying activity of the pathological process. These sera were positive in the collodion agglutination with HA in 59% of the examined sera, i.e. the titre was 1:4 and higher. Most frequently achieved titres were 1:8 up to 32.

In the latter group we compared the results achieved with HA as antigen with the results of the other nonpurified tissue antigens (connective tissue, skin, myocardium, brain, bone-marrow, liver, spleen). On account of the negative controls in the former group, it was possible to combine the results in two groups to get greater numbers: one with negative results, the other with positive results. The dependence was appraised by the χ^2 -test. The results are shown in the Table.

The hypothesis of independence between HA- and organ-antibodies was disproved for myocardium, con-

with an other nonpurified connective antigen show, however, that even other components than HA can be affected by the immunotoxic mechanism in collagen diseases.

At present, however, no definite statement can be made as to what kind of hyaluronic acid acts as the primary antigen in autoimmunization in rheumatism—the hyaluronic acid originating in the tissue proper or that originating in the streptococcus-capsules. It cannot be also stated whether HA-antibodies cause changes that are manifested e.g. by precipitation by being bound to tissue HA. The role of autoantibodies in the development of fibrinoid necrosis presents at present only a working hypothesis awaiting its confirmation by model tests.

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Plzeň, Kollárova 1, Československo, April 11, 1957.

Zusammenfassung

In Seren *Febris rheumatica*-Kranker wurden Autoantikörper gegen isolierte Hyaluronsäure durch Kollodion-Agglutination gefunden.

⁴ D. McCLEAN, H. J. ROGERS, and B. W. WILLIAMS, *Lancet* 244, 355 (1943).

⁵ S. HARRIS and T. N. HARRIS, *Amer. J. med. Sci.* 217, 174 (1949).

⁶ P. A. CAVELTI, *J. Immunol.* 57, 141 (1947).

⁷ V. WAGNER, V. REJHOLEC, Z. MANDLÍKOVÁ, and M. KRÍŽOVÁ, *Čsl. Biol.* 1, 376 (1952). — V. WAGNER and V. REJHOLEC, *Ann. Rheum. Dis.* 15, 364 (1956).

⁸ CH. H. ALTSHULER and D. M. ANGEVINE, *Amer. J. Path.* 27, 141 (1951).

* Statistical evaluation: V. MALÝ. Technical assistance: ALENA SOVÁKOVÁ.

Pharmakologische Charakterisierung von synthetischem Hypertensin

Hypertensin (Angiotonin), das Reaktionsprodukt der Einwirkung von Renin auf Hypertensinogen (BRAUN-MENENDEZ *et al.*¹, PAGE und HELMER², liegt in Form eines Dekapeptids (Hypertensin I) und eines Oktapeptids (Hypertensin II) vor (SKEGGS *et al.*³, ELLIOTT und PEART⁴). Kürzlich gelang es SCHWYZER und Mitarbeitern, Hypertensin I, Hypertensin II sowie einige nahe verwandte Peptide erstmalig auf synthetischem Wege darzustellen⁵. Die pharmakologische Untersuchung ergab eine weitgehende Übereinstimmung der Wirkung der synthetisierten Peptide mit extraktiv gewonnenem Hypertensin. Von dem wahrscheinlich mit Rinderhypertensin II identischen Val₅Oktapeptid (RH II) stand eine grössere Menge zur Verfügung, so dass es möglich war, den pharmakologischen Wirkungstypus dieser Substanz im Vergleich zu anderen blutdrucksteigernden Stoffen und im Hinblick auf seine antagonistische Beeinflussbarkeit genauer zu untersuchen und mit Befunden zu vergleichen, die früher mit natürlichem Hypertensin erhoben wurden (MEIER *et al.*⁶).

a) *Wirkung am Rattenblutdruck.* Die blutdrucksteigernde Wirkung wurde an der Ratte untersucht, und zwar am intakten Tier, nach Vorbehandlung mit Ganglienblockern und am bilateral nephrektomierten Tier. An der mit Urethan narkotisierten intakten Ratte führt RH II zu einem gleich hohen und gleich langen Blutdruckanstieg wie nor-Adrenalin. Die durchschnittliche Blutdrucksteigerung nach Injektion von 1 γ /kg betrug 25 mm Hg, und nach etwa 2,5 min war der Blutdruck auf den Ausgangswert zurückgekehrt.

Entsprechend der von PEART⁷ angegebenen Methode wurde RH II an der mit ganglienblockierenden Substanzen vorbehandelten Ratte vergleichend mit nor-Adrenalin auf seine pressorische Wirkung untersucht. Als Ganglienblocker fanden Ansolysen in der von PEART gegebener sehr hohen Dosis von 25 mg/kg subkutan, sowie Ecolid in einer Dosis von 0,1 mg/kg intravenös Verwendung. Nach dieser Vorbehandlung zeigt RH II in Dosen von 0,5–4 γ /kg eine etwa gleich intensive und gleich lange Blutdruckzunahme wie gleiche Dosen von nor-Adrenalin (Abb. 1).

An der nierenlosen Ratte ist die drucksteigernde Wirkung von Hypertensin im Vergleich zum intakten Tier verstärkt und verlängert⁸. Bei Testierung an Tieren, die 20 h vor Injektion nephrektomiert waren, ruft RH II in Dosen von 1 γ /kg eine Drucksteigerung bis zu

60 mmHg hervor, die nach etwa 5 min auf den Ausgangswert zurückgeht (Abb. 2).

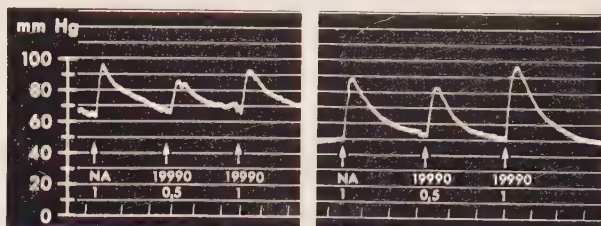


Abb. 1. Ratte, Urethannarkose. Carotisdruck.

1 γ /kg nor-Adrenalin, 0,5 und 1,0 γ /kg RH II (Nr. 19990) intravenös, links: vor, rechts: nach intravenöser Injektion von 0,1 mg/kg Ecolid.

Durch Dauerinfusion von RH II (0,1 mg/kg während 30 min) lässt sich an der nierenlosen Ratte eine allmählich einsetzende, anhaltende Blutdruckerhöhung um 25–30 mm Hg hervorrufen. Nach Absetzen der Infusion geht der Blutdruck innerhalb von 2–4 min auf seinen Ausgangswert zurück. Intensität und Dauer der akuten pressorischen Wirkung nach einmaliger Injektion und Drucksteigerung bei Dauerinfusion sind bei synthetischem RH II und bei natürlichem Hypertensin (Angiotonin⁹) qualitativ gleich.

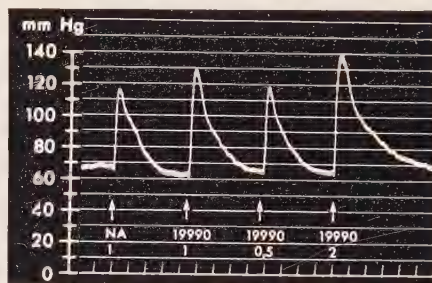


Abb. 2. Bilateral nephrektomierte Ratte, Urethannarkose. Carotisdruck.

1,0 γ /kg nor-Adrenalin, 1,0 γ /kg, 0,5 γ /kg, 2,0 γ /kg RH II (Nr. 19990) intravenös.

Durch Erhitzen (30 min auf 100°) wird die pressorische Wirkung von RH II nicht beeinträchtigt. Dagegen zerstört frisch hergestellter Nierenextrakt den drucksteigernden Effekt. Auch in dieser Hinsicht verhält sich synthetisch hergestelltes Hypertensin gleich wie natürliches.

b) *Antagonistische Beeinflussung der Blutdrucksteigerung.* Die drucksteigernde Wirkung von RH II wird an der intakten Ratte durch Sympathicolitika (Regitin) nicht beeinflusst. Dagegen führt Hydralazin (Apresolin (0,25 mg/kg)) zu einer leichten Abschwächung des Druckanstieges. Chlorpromazin (0,1 mg/kg) und Reserpin (1 mg/kg) sind ohne Einfluss.

An der nierenlosen Ratte lässt sich nach Vorbehandlung mit Regitin, Chlorpromazin, Reserpin und Apresolin eine deutliche Hemmung der pressorischen Wir-

¹ E. BRAUN-MENENDEZ, J. C. FASCILO, L. F. LOIR und J. M. MUNOZ, *Rev. Soc. argent. Biol.* 15, 420 (1939). – BRAUN-MENENDEZ, *Pharmacol. Rev.* 8, 25 (1956).

² J. H. PAGE und O. H. HELMER, *J. exp. Med.* 71, 495 (1940).

³ L. T. SKEGGS, J. R. KAHN und N. P. SHUMWAY, *J. exp. Med.* 103, 295 (1956). – L. T. SKEGGS, K. E. LUTZ, J. R. KAHN, N. P. SHUMWAY und K. R. WOODS, *J. exp. Med.* 104, 193 (1956).

⁴ D. F. ELLIOTT und W. S. PEART, *Nature* 177, 527 (1956).

⁵ W. RITTEL, B. ISELIN, H. KAPPELER, B. RINIKER und R. SCHWYZER, *Helv. chim. Acta* 40, 614 (1957). – W. RITTEL, B. ISELIN, H. KAPPELER, B. RINIKER und R. SCHWYZER, *Angew. Chemie* 69, 179 (1957).

⁶ R. MEIER, H. J. BEIN, F. GROSS und J. TRIPOD, *Proceedings of the IIIrd International Congress for International Medicine* (Stockholm 1954).

⁷ W. S. PEART, *Biochem. J.* 59, 300 (1955).

⁸ F. GROSS und F. SULSER, *Arch. exp. Path. Pharm.* 229, 338 (1956).

⁹ Als Vergleichspräparat benutzten wir ein uns von Herrn Dr. SCHWARZ, Cleveland Clinic, Cleveland, Ohio, zur Verfügung gestelltes, weitgehend gereinigtes Angiotonin, von dem etwa 50 mal höhere Dosen gegeben werden mussten, um einen gleichen Druckanstieg wie mit RH II zu erzielen.

kung erzielen, so dass sich das nierenlose Tier in dieser Hinsicht anders verhält als das intakte Tier. Dagegen verstärken Ganglienblocker die Drucksteigerung von RH II am nierenlosen Tier mehr als am intakten Tier. Die durch Dauerinfusion von RH II ausgelöste Blutdruckzunahme wird sowohl am intakten als auch am nierenlosen Tier durch Vorbehandlung mit Ecolid verstärkt. Im Gegensatz dazu senkt Ecolid in gleicher Dosis den Blutdruck, wenn es während der Infusion mit RH II injiziert wird, wobei die Intensität des Druckabfalls von der Höhe des Ausgangsdruckes abhängt.

c) *Wirkung und antagonistische Beeinflussung von RH II an isoliert durchströmten Gefässen.* An den isoliert durchströmten Gefässen der Hinterextremität des Kaninchens führt RH II zu einer starken Vasokonstriktion. Eine 50prozentige Verminderung der Durchflussmenge war mit Konzentrationen von 10^{-8} (g/ml) zu erzielen. Adrenalin zeigt in dieser Versuchsanordnung eine 10fach stärkere Wirkung und bewirkt in einer Konzentration von 10^{-9} (g/ml) eine gleiche Abnahme der Perfusionsgrösse.

Durch Phentolamin (Regitin (10^{-5} g/ml) lässt sich die Wirkung von RH II nicht beeinflussen. Dagegen hemmen Chlorpromazin (3×10^{-6}), Reserpin (10^{-6}) und Dihydralazine (Nepresol (10^{-5})) die durch RH II (10^{-8}) hervorgerufene Verminderung der Durchflussmenge um 50%. Ganglienblocker sind bis zu hohen Konzentrationen wirkungslos.

Am isolierten Kaninchen- und Katzenherzen (LANGENDORFF) führen Konzentrationen von 10^{-9} (g/ml) zu Verengung der Coronargefässe mit gleichzeitiger geringgradiger Zunahme der Amplitude und der Herzfrequenz, deren Beeinflussbarkeit durch Antagonisten geprüft wird.

In noch nicht abgeschlossenen Untersuchungen fand sich, dass das dem RH II entsprechende Ileu₅ Oktapeptid eine etwa 2–3mal stärkere Wirkungsintensität in verschiedenen Versuchsanordnungen besitzt. Diese Wirkungssteigerung ist wahrscheinlich auf einen höheren Reinheitsgrad zurückzuführen.

R. MEIER, F. GROSS, J. TRIPOD
und H. TURRIAN

Aus den Forschungslaboratorien der CIBA A.G.,
Basel, Pharmazeutische Abteilung, 15. Juni 1957.

Summary

Synthetic hypertensin, RH II, a Val₅octapeptide shows the same pattern of activity as natural hypertensin (angiotonin). In the normal rat, and in the rat pretreated with ganglionic blocking substances, the hypertensive activity corresponds to that of nor-adrenaline while it is enhanced in the nephrectomised animal. In the intact animal, hydralazine diminishes the hypertensive effect, while Regitin, Chlorpromazine and Reserpine are without effect. On the perfused vessels of the rabbit's hind limb, RH II produces a vasoconstriction which is diminished by Chlorpromazine and hydralazine, but not by Regitin. RH II is not destroyed by heating, but by freshly prepared kidney extract.

Colorants acides et détermination embryonnaire chez les Echinodermes

Chez les Echinodermes, le déplacement de l'équilibre entre les territoires présomptifs entomésodermiques d'une part et ectodermiques d'autre part, en faveur de ces derniers, a reçu le nom d'animalisation. Ce phénomène a fait l'objet de nombreux travaux, HÖRSTADIUS¹ LINDAHL².

Nous avons montré précédemment³ que les dérivés sulfoniques acides exercent une action animalisante sur ledéveloppement de l'œuf de l'oursin *Paracentrotus lividus*. Toutes les substances actives étudiées bien qu'appartenant à des séries chimiques distinctes: colorants azoïques, colorants dérivés du triphénylméthane, polyamines complexes, polyphénols, présentent en commun des propriétés acides marquées dues à la présence de groupes sulfoniques. Nous avons été ainsi conduits à établir une relation entre les effets animalisants de ces substances et leurs propriétés acides, et à prendre en considération le rôle des combinaisons formées entre ces substances et les éléments basiques intracellulaires, les protéines notamment, dans le déterminisme de l'animalisation.

Les groupes carboxyles présentant pour les protéines une affinité voisine de celle des groupes sulfoniques (KLOTZ⁴), on pouvait s'attendre dans ces conditions à observer des effets animalisants avec des substances acides portant des groupes carboxyles. Pour vérifier cette hypothèse, nous avons examiné trois colorants acides carboxylés. Nous indiquons pour chacun d'eux son numéro de classification dans le «Colour Index». L'uranine (N° 766) et le rose Bengale (N° 779) sont des colorants dérivés du xanthène. Le violet de chrome CG (N° 727) est un dérivé de l'acide pararosolique appartenant à la série du triphénylméthane.

Les œufs de l'oursin *Paracentrotus lividus* sont mis 30 min après la fécondation dans les solutions de colorants dans l'eau de mer, où ils sont laissés pendant toute la durée de la culture. Aux concentrations élevées, les colorants inhibent l'éclosion et celle-ci ne peut être obtenue qu'après le transfert des embryons dans l'eau de mer normale. Nous avons figuré dans un tableau les types d'embryons observés. Ce sont des embryons munis d'un champ cilié et pourvus ou non d'une petite vésicule archentérique (a, b), des blastulas hyperciliées (c, d) de type $\frac{3}{4}$ ou $\frac{1}{2}$ selon la classification de HÖRSTADIUS, évoluant ultérieurement en blastulas permanentes entièrement recouvertes de cils courts. Des formes radialisées (e, f, g) sont également observées.

MOORE⁵ ayant signalé les effets toxiques de la lumière sur les cultures en présence de certains colorants, nous avons examiné la participation éventuelle de la lumière dans la production expérimentale de l'animalisation par le rose Bengale. Le séjour à l'obscurité prolonge quelque peu la vie des embryons traités, mais leur animalisation reste du même type que celle des embryons laissés à la lumière. Nous pouvons donc conclure que la lumière n'intervient pas au cours de l'animalisation par le rose Bengale.

Les colorants étudiés se montrent donc d'actifs agents modificateurs de la détermination embryonnaire. Selon

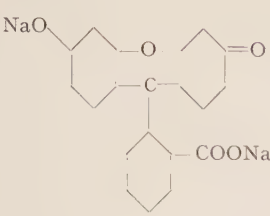
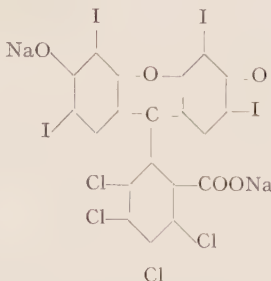
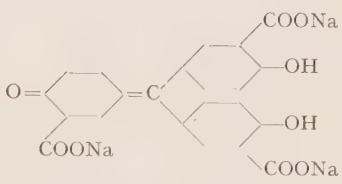
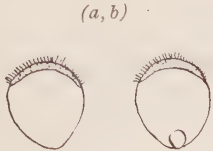
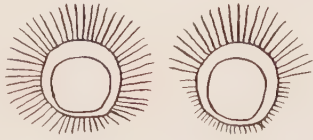
¹ S. HÖRSTADIUS, Pubbl. Staz. zool. Napoli 14, 251 (1935).

² P. E. LINDAHL, Acta zool. Stockholm 17, 179 (1936).

³ R. LALLIER, Exp. Cell Res. 9, 232 (1955); C. r. Acad. Sci. 242, 2772 (1956).

⁴ I. KLOTZ, J. Amer. chem. Soc. 68, 2299 (1946).

⁵ A. R. MOORE, Arch. Sci. biol. 12, 231 (1928).

Uranine (N° 766)	Rose bengale (N° 779)	Violet de chrome CG (N° 727)
		
 1/600	 1/5000, 1/10000	 1/10000
 1/5000	 1/50000	

la concentration, ils sont animalisants, ou bien ils orientent le développement vers un type d'organisation à symétrie radiale. Nous retrouvons ici un phénomène déjà observé avec d'autres agents animalisants acides, à savoir le passage des formes radialisées aux formes animalisées, au fur et à mesure que la concentration de l'agent animalisant augmente⁶. Lorsque la concentration est suffisante, ces colorants sont en outre capables d'inhiber ou de retarder l'éclosion. Il s'agit là toutefois d'un phénomène dont le déterminisme apparaît distinct, ainsi que nous l'avons montré dans un travail antérieur³, en étudiant des cultures mixtes comprenant un agent animalisant et un agent végétativisant, le chlorure de lithium.

En examinant les particularités structurales de ces trois colorants, on note tout d'abord que le violet de chrome CG est un dérivé du triphénylméthane tandis que l'uranine et le rose Bengale sont des dérivés du Xanthène. Dans ces expériences ces différences structurales n'apparaissent pas intervenir dans l'activité de ces substances. La très faible solubilité du violet de chrome CG dans l'eau de mer ne permet pas d'explorer son activité à des concentrations supérieures à 1/10 000. Examinons maintenant les propriétés communes à ces trois colorants. Substances acides, elles doivent cette propriété à la présence d'un groupe carboxyle (uranine, rose Bengale) ou de trois groupes carboxyles (violet de

chrome CG). Le rose Bengale étroitement apparenté à l'uranine en diffère par la fixation d'atomes de chlore et d'iode sur sa molécule. Or, une règle chimique classique indique que la présence de substituants électro-négatifs accroît la force de la fonction acide et ce phénomène est d'autant plus marqué que les substituants sont plus électro-négatifs, qu'ils sont plus nombreux et plus proches du groupe acide. La présence dans la molécule de rose Bengale de quatre atomes d'iode et de quatre atomes de chlore lui confère donc une acidité plus forte que celle de l'uranine qui ne porte aucun substituant électro-négatif. La comparaison entre les types d'embryons obtenus et les concentrations utilisées mettant nettement en évidence la supériorité de l'activité animalisante du rose Bengale sur celle de l'uranine, il est intéressant de rapprocher ces données de celles obtenues en comparant les propriétés acides de ces deux colorants. On peut ainsi en déduire que l'activité animalisante de ces agents est étroitement liée à la force de leur fonction acide. Celle-ci gouvernant leur affinité pour les éléments basiques du cytoplasme, l'étude des combinaisons formées apparaît ainsi susceptible d'apporter quelques précisions sur le mécanisme de l'animalisation et d'une façon plus générale sur les processus de la différenciation cellulaire.

R. LALLIER

Institut de Biologie physico-chimique, Paris, et Station zoologique, Villefranche-sur-Mer, le 11 avril 1957.

⁶ R. LALLIER, Exper. 12, 217 (1956).

Summary

The effects of three dyes with carboxyl groups are been studied on the developing eggs of the sea urchin, *Paracentrotus lividus*. Uranin and rose Bengal are both some derivative of the xanthene. Chrome violet CG is a carboxyl derivative of pararosolic acid. In dilute solutions these three dyes induce the development of radial larvae. At higher concentration, uranin and rose Bengal are very effective animalizing agents. Rose Bengal is more active than uranin. Rose Bengal differs from uranin by the fixation of halogens on the molecule of dye which increase the acidity of the carboxyl group. It appears that animalizing activity runs parallel with acidity. The results of these experiments are discussed in relation to the animalizing effects of various acid sulfonated dyes. The possible significance of the combinations between acid dyes and basic components of the cells in the animalization is considered.

Über die Stoffwechselwirkung eines Lipopolysaccharids aus *Escherichia coli*

Zahlreiche Mitteilungen der letzten Jahre weisen darauf hin, dass eine Injektion von Bakterienlipopolysacchariden Wirkungen hervorruft, die weit über den Temperaturanstieg hinausgehen¹. Dabei ist aber auch der Mechanismus der Temperaturerhöhung selbst bei weitem noch nicht geklärt.

In unseren Versuchen befassten wir uns mit der eventuellen Stoffwechselwirkung eines Lipopolysaccharids, das wir aus *E. coli*, Stamm ATCC Nr. 9492, gemäss dem Verfahren nach WESTPHAL *et al.*² isolierten. Die minimale

¹ E. EICHENBERGER, M. SCHMIDHAUSER-KOPP, H. HURNI, M. FRICISAY und O. WESTPHAL, Schweiz. med. Wschr. 85, 1190, 1213 (1955). – R. MEIER, H. J. BEIN und R. JAUQUES, Exper. 12, 235 (1956). – E. FRITZE, P. DOERING, H. MANECKE und R. SCHOEN, Schweiz. med. Wschr. 83, 783 (1953). – D. A. MCGINTY, M. MCWILSON und G. RODNEY, Proc. Soc. exp. Biol. Med. 70, 334 (1949).

² O. WESTPHAL, O. LÜDERITZ, E. EICHENBERGER und W. KEIDERLING, Z. Naturf. 7B, 536 (1952).

pyrogene Dosis dieses Stoffes beträgt für Kaninchen (intravenös) 0,0042 γ /kg und die DL₅₀ für Mäuse (intravenös) 9,9 mg/kg.

Methodik. Die Gewebsatmung von Schnitten aus Kaninchenleber und Niere wurde im Warburgapparat untersucht. Als Medium haben wir entweder Krebs-Ringer-Lösung mit Glukose oder homogenes Serum benutzt. Auch das Lipopolysaccharid wurde in diesen Medien gelöst. Falls Serum benutzt wurde, inkubierten wir die Pyrogenlösung vor dem eigentlichen Experiment 1 h bei 37°. Die Menge des Lipopolysaccharids, die in den Seitenarm des Gefässes kam, betrug 0,002 γ . In einer anderen Versuchsreihe wurde das Pyrogen den Kaninchen injiziert (0,01 γ /kg intravenös), die Tiere nach 1 1/2 h bei hohem Fieber getötet und der Sauerstoffverbrauch der Leber bestimmt. Bei anderen Tieren wurde der Blutzuckergehalt nach der Methode von KING und GARNER³ und die Blutmilchsäure nach SUMMERSON und BARKER⁴ vor und nach der Pyrogenverabreichung gemessen.

Resultate. Die genauen Daten über die Wirkung auf den Glukose- und Milchsäuregehalt sind der Tabelle I zu entnehmen.

Tabelle I

Einfluss von Pyrogen auf den Blutzucker- und Milchsäuregehalt im Kaninchenvenenblut

Versuchsanordnung	Zahl der Tiere	Gehalt		<i>p</i>
		vor	nach Pyrogen	
Glukose in mg%	30	93,6 $\pm$ 8,9	120 $\pm$ 9,7	<i>p</i> < 0,02
Milchsäure in mg%	30	42,9 $\pm$ 4,2	25 $\pm$ 3,3	<i>p</i> < 0,02

Tabelle II stellt die Wirkung auf den Sauerstoffverbrauch der Leber und der Niere dar.

³ E. J. KING, *Microanalysis in Medicine and Biochemistry* (London 1951).

⁴ S. BARKER und W. H. SUMMERSON, J. biol. Chem. 138, 535 (1941).

Tabelle II

Wirkung von Pyrogen auf den Sauerstoffverbrauch der Leber- und Nierenschnitte

Nr.	Versuchsanordnung	Zahl der Versuche	O ₂ -Verbrauch in Mikroliter pro 1 mg Trockengewicht während 2 h	<i>p</i>
1	Leber, Kontrolle im Krebs-Ringer	24	1,59 $\pm$ 0,07	
2	Leber + 0,002 γ Pyrogen in Krebs-Ringer	31	1,50 $\pm$ 0,1	<i>p</i> gegenüber Versuch 1 < 0,2
3	Leber, Kontrolle im Serum	24	1,82 $\pm$ 0,08	<i>p</i> gegenüber Versuch 1 < 0,01
4	Leber + 0,002 γ Pyrogen im Serum inkubiert	20	2,18 $\pm$ 0,11	<i>p</i> gegenüber Versuch 1 < 0,01 <i>p</i> gegenüber Versuch 3 < 0,02
5	Leber von Kaninchen, denen 0,01 γ /kg Pyrogen injiziert wurde, nach 1 1/2 h getötet	32	2,55 $\pm$ 0,05	<i>p</i> gegenüber Versuch 1 < 0,001 <i>p</i> gegenüber Versuch 3 < 0,01
6	Niere, Kontrolle im Krebs-Ringer	20	6,19 $\pm$ 0,17	
7	Niere, Kontrolle im Serum	25	7,24 $\pm$ 0,12	<i>p</i> gegenüber Versuch 6 < 0,001
8	Niere + Pyrogen 0,002 γ im Serum inkubiert	26	6,55 $\pm$ 0,12	<i>p</i> gegenüber Versuch 7 < 0,01

Besprechung der Ergebnisse. Mehrere Forscher befassten sich mit der Frage, ob der Temperaturanstieg nach bakteriellen Pyrogenen primär auf einer Verminderung der Wärmeabgabe oder auf einer Stoffwechselsteigerung beruht⁵. Die schon von anderen Autoren beschriebene⁶, hyperglykämisierende Wirkung der Pyrogene sowie die Senkung des Milchsäurespiegels sind statistisch signifikant und weisen auf eine Aktivierung des Stoffwechsels hin. Es ist dabei an die Arbeit von HAMRICK *et al.*⁷ zu erinnern, in welcher über eine Beschleunigung des Stoffwechsels im Splanchnicusgebiet berichtet wird.

Unsere in dieser Arbeit mitgeteilten Untersuchungen zeigen, dass der Sauerstoffverbrauch der Leber nach Pyrogengaben signifikant vergrößert wird und zwar nicht nur *in vivo*, was unter Umständen ein Sekundäreffekt sein könnte, sondern auch *in vitro*, was mit Sicherheit auf einen primären Angriffspunkt hinweist. Besonders interessant erscheint uns, dass der Sauerstoffmehrerverbrauch nur stattfindet, wenn Serum mit Pyrogenen inkubiert wurde. Wir vermuten, dass, wie schon andere Forscher annahmen⁸, die Pyrogene an Bluteiweisskörper gebunden werden und damit die eigentlichen, endogenen Pyrogene entstehen. Das Fehlen einer ähnlichen Wirkung auf die Niere oder sogar eine Verminderung der Atmung ist vorläufig schwer zu erklären. Die von uns erzielten Resultate weisen in der Richtung, dass der Stoffwechselsteigerung eine wesentliche und primäre Rolle beim Temperaturanstieg zukommt.

J. VENULET und ANNA DESPERAK

Anstalt für Pharmakologie, Arzneimittelforschungsinstitut, Warschau, den 3. Juni 1957.

Summary

It has been established that pyrogen causes hyperglycemia and diminution of the lactic acid level in the blood. In *in vitro* investigations, oxygen requirements of the liver slices show marked increase. The results are regarded as showing a primary action of the pyrogens on metabolism.

⁵ E. EICHENBERGER, M. SCHMIDHAUSER-KOPP, H. HURNI, M. FRICSAJ und O. WESTPHAL, Schweiz. med. Wschr. 85, 1213 (1955).
⁶ R. GRANT und W. J. WHALEN, Amer. J. Physiol. 173, 47 (1955).
⁷ H. SIEDEK und H. HÄUSLER, deutsch. med. Wschr. 80, 1128 (1955).

⁸ P. B. BEESON, J. exper. Med. 86, 29 (1947).

⁷ L. W. HAMRICK jr. und J. D. MYERS, J. Lab. clin. Med. 45, 568 (1955).

⁸ R. GRANT und W. J. WHALEN, Amer. J. Physiol. 173, 47 (1955).
 – V. MENKIN, J. Lab. clin. Med. 46, 423 (1955).

Über die Hemmung des aktiven Kationentransportes durch Herzglykoside

Aus den Untersuchungen von FLECKENSTEIN *et al.*¹ geht hervor, dass bei Hemmung des aktiven Kationentransportes durch Zellmembranen unter der Einwirkung von Atmungs-, Glykolyse- und Phosphorylierungsgiften gleichzeitig eine Erschöpfung des Zellvorrates an energiereichem Phosphat (Adenosintriphosphat,

Kreatinphosphat) eintritt. Da die Herzglykoside und deren Aglykone den aktiven Kalium-Natrium-Austausch durch die Erythrozytenmembran blockieren², wurde zur weiteren Klärung ihres Wirkungsmechanismus die Frage geprüft, ob auch diese Hemmung auf eine energetische Insuffizienz der Zelle zurückzuführen ist.

Methoden. Menschliches defibriniertes Frischblut oder «Kälteblut» (nach HARRIS³ 6 Tage bei 4°C aufbewahrt) wurden nach Zusatz von Glucose (500 mg%) und Testsubstanzen in gleichem Gesamtvolumen aller Ansätze (4,8 ml Blut + 0,2 ml Lösungsmittel) bei 37°C inkubiert. Nach der Inkubation wurde das Blut zentrifugiert und das Serum durch Absaugen entfernt. Nach zweimaligem Waschen mit isotonischer MgCl₂-Lösung wurden die Erythrozyten mit quartzdestilliertem Wasser hämolytisiert und der Kalium- und Natriumgehalt des Hämolyсата flammenphotometrisch bestimmt. Ausserdem wurde für jede Inkubationszeit der Hämatokritwert gemessen. Die Analyse der energiereichen Phosphatverbindungen erfolgte nach dem von FLECKENSTEIN *et al.*⁴ angegebenen Verfahren, wobei die Erythrozyten von 1 ml Blut bei 0°C nach Abzentrifugieren des Serums zweimal mit isotonischer NaCl-Lösung gewaschen und anschliessend 10 min mit 1,5 ml 5%iger Trichloressigsäure extrahiert wurden. Eine weitere Modifikation bestand darin, dass bei der Chromatographie nach dem Trennungsgang im ersten Lösungsmittel das Papier in grösserem Abstand (8 cm) von der Startlinie abgeschnitten wurde. Dadurch konnte die 2,3-Diphosphoglycerinsäure (2,3-DPGS) nach Anwendung des zweiten Lösungsmittels ebenfalls lokalisiert und bestimmt werden.

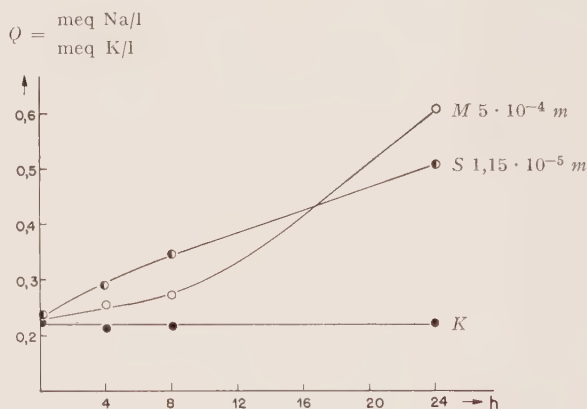


Abb. 1. Hemmung des aktiven Kationentransportes bei Inkubation von Frischblut. Ordinate: Q gibt das Verhältnis Natriumgehalt/Kaliumgehalt der Erythrozyten an, gemessen in Milliäquivalenten pro Liter Zellen. Abszisse: Inkubationszeit in Stunden. K = Kontrollansatz, M = Monojodacetat $5 \cdot 10^{-4} m$, S = k -Strophanthosid $1,15 \cdot 10^{-5} m$ ($10^{-5} g/ml$).

Resultate. Bei der Inkubation von Frischblut (Abb. 1) bleibt der normale Elektrolytgehalt der Erythrozyten während 24 h unverändert erhalten. Durch aktiven Transport wird also dauernd Natrium nach aussen und Kalium nach innen befördert. Bei Hemmung des aktiven Kationentransportes durch Monojodessigsäure (MJE) oder k -Strophanthosid überwiegen die «passiven» Ionenverschiebungen entsprechend dem Konzentrations-

² H.-J. SCHATZMANN, Helv. physiol. Acta 11, 346 (1953).

³ J. E. HARRIS, J. biol. Chem. 141, 579 (1941).

⁴ A. FLECKENSTEIN, Der Kalium-Natrium-Austausch (Springer-Verlag, Berlin 1955). – E. GERLACH, Arch. exp. Path. Pharmacol. 228, 128 (1956). – A. FLECKENSTEIN, E. GERLACH und J. JANKE, Schweiz. med. Wschr. 86, 1041 (1956).

Tabelle I

Inkubation von Frischblut. Gleicher Versuch wie Abbildung 1 und 2. Die Werte sind in γ /ml Erythrozyten angegeben. Übrige Angaben siehe Abbildung 1 und 2.

	K_0	M_0	S_0	K_8	M_8	S_8	K_{24}	M_{24}	S_{24}
ATP	171	179	185	212	—	227	238	—	249
ADP	96	94	89	22	24	15	—	—	—
2,3-DPGS	585	500	552	542	28	525	69	—	77
Anorganischer P (H_3PO_4) .	13	20	17	16	180	19	219	330	204

Tabelle II

Inkubation von « Kälteblut ». Gleicher Versuch wie Abbildung 3. Die Werte sind in γ /ml Erythrozyten angegeben. Übrige Angaben siehe Abbildung 1 und 2.

	K_0	M_0	S_0	K_4	M_4	S_4	K_8	M_8	S_8
ATP	104	98	113	82	—	86	95	—	87
ADP	42	37	47	—	—	—	—	—	—
2,3-DPGS	973	956	930	552	573	570	565	156	551
Anorganischer P (H_3PO_4) .	59	62	54	153	242	139	171	314	152

gradienten zwischen Serum und Erythrozyten, was sich in einer Erhöhung des intrazellulären Natriums und einer Abnahme des Kaliums äussert (Zunahme von Q). Aus Abbildung 2 und Tabelle I ist ersichtlich, dass der Stoffwechsel des energiereichen Phosphats in den Erythrozyten nur durch MJE, nicht aber durch das Herzglykosid beeinflusst wird. Innerhalb der Genauigkeit der Methode besteht eine völlige Übereinstimmung zwischen Kontroll- und k -Strophanthosidansätzen: der Gehalt an Adenosintriphosphat (ATP) steigt im Laufe

der Inkubation über 24 h sogar noch an, während er unter der Einwirkung von MJE nach 8 h auf Null abgesunken ist. Ebenso verschwindet die 2,3-DPGS in den MJE-Ansätzen schneller, da sie infolge der Hemmung der Oxydation des Phosphoglycerinaldehyds durch MJE gar nicht mehr gebildet wird. Nach 24 h sind aber auch in den Kontrollansätzen nur noch Spuren von 2,3-DPGS – bei gleichzeitig erhöhtem ATP-Gehalt – vorhanden. Dieser Befund zeigt übrigens mit aller Deutlichkeit, dass der aktive Kationentransport der Erythro

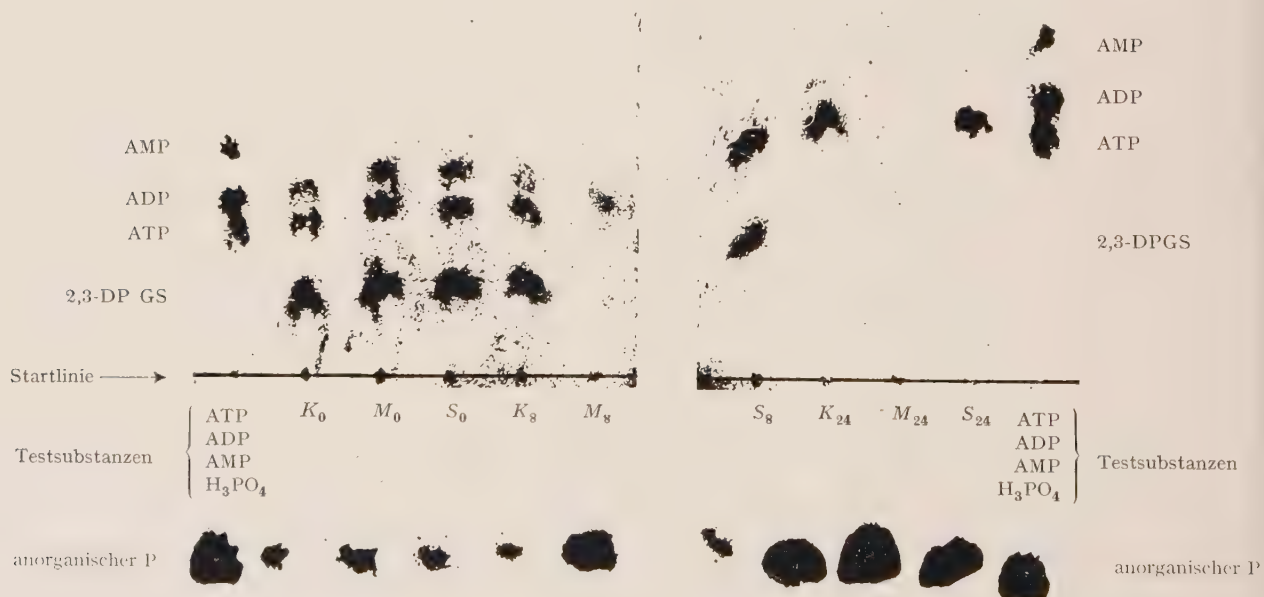


Abb. 2. Chromatogramme der Erythrozyten-Extrakte nach Entwicklung mit Molybdatreagens nach HANES und ISHERWOOD⁵. Gleicher Versuch wie Abbildung 1. Die Indices von K , M und S geben die Inkubationsdauer in Stunden an. ATP = Adenosintriphosphat, ADP = Adenosindiphosphat, AMP = Adenosinmonophosphat, 2,3-DPGS = 2,3-Diphosphoglycerinsäure. Übrige Angaben siehe Abbildung 1.

⁵ C. S. HANES und F. A. ISHERWOOD, Nature 164, 1107 (1949).

zyten in unmittelbarer Weise vom ATP abhängt⁶. Das anorganische Phosphat steigt beim Verschwinden der organischen Phosphorverbindungen entsprechend an.

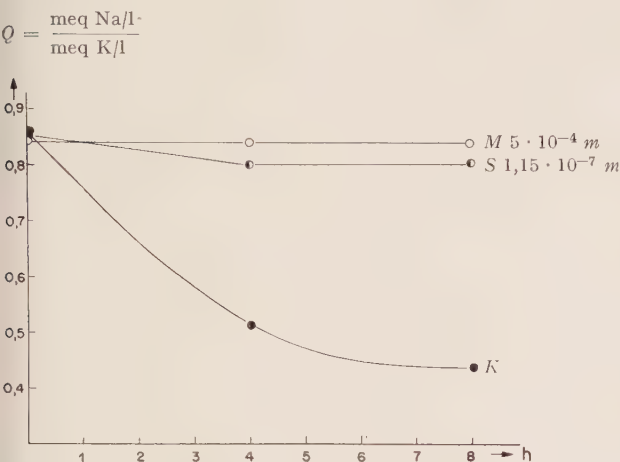


Abb. 3. Hemmung des aktiven Kationentransportes bei Inkubation von «Kälteblut» (gleiches Blut wie Versuch der Abbildung 1, 6 Tage bei 4°C aufbewahrt). $S = k$ -Strophanthosid $1,15 \cdot 10^{-7} \text{ m}$ (10^{-7} g/ml). Übrige Angaben siehe Abbildung 1.

Bei Inkubation von «Kälteblut» (Abb. 3) wird die normale Kationenverteilung zwischen Zellen und Serum durch aktiven Transport wieder hergestellt, das heisst, der anfänglich hohe Quotient Q der relativ natriumreichen und kaliumarmen «Kälteerythrozyten» fällt im Laufe der Inkubation ab und nähert sich wiederum dem «Normalwert». In Gegenwart von MJE oder k -Strophanthosid bleibt dieser Abfall von Q aus. Auch beim «Kälteblut» lässt sich keine Wirkung des Herzglykosids auf den Phosphatstoffwechsel nachweisen (Tab. II): ATP verschwindet wiederum nur in den MJE-Ansätzen. Die «Kälteerythrozyten» enthalten weniger ATP und Adenosindiphosphat (ADP), hingegen mehr 2,3-DPGS und anorganisches Phosphat. Ebenso nimmt der Gehalt von 2,3-DPGS bei Zusatz von MJE schneller ab.

MJE und Herzglykoside greifen also an zwei ganz verschiedenen Stellen in den Ablauf der Reaktionen ein, welche dem aktiven Transport zugrunde liegen. Die vorliegenden Befunde weisen darauf hin, dass die Hemmung des aktiven Kalium-Natrium-Austausches durch Herzglykoside nicht auf einer Blockierung energie liefernder Vorgänge, sondern auf einer direkten Wirkung auf den Membrantransportmechanismus, das «Carrier-System», beruht.

Beim Abschluss der Arbeit erhielten wir Kenntnis vom Autorreferat von R. WHITTAM für das Programm der Englischen Physiologentagung vom 29. bis 30. März 1957, auf welcher er über ähnliche Untersuchungsergebnisse berichtet hat.

H. A. KUNZ und F. SULSER

Pharmakologisches Institut der Universität Bern, 31. Mai 1957.

Summary

Inhibition of active cation transport through the red cell membrane by cardiac glycosides is not accompanied

by alteration of phosphate metabolism (ATP, ADP, 2,3-diphosphoglyceric acid) in contrast to the action of iodoacetate. This fact suggests that the cardiac glycosides act directly on the membrane transport mechanism.

Studies of Lipid Extracts from Red Blood Cells

A lipid extract obtained from Rh positive human red blood cells was reported by the writer in 1947¹ and other findings have been published since². Recently a study³ of the specificity of such an extract was made by means of quantitative nitrogen assays of Rh antibody adsorbed on to the lipid particle. It has been suggested⁴ that the amount of specific substance produced by the methods employed is so slight as to be negligible. Since other findings tend to refute this⁵, it was resolved to investigate use of identical methods with other than human red cells in order to see if comparable specific material could be derived by the same techniques.

Accordingly, it was decided to choose for this purpose beef red cells and to attempt to extract from them the lipid-carbohydrate complex which has been characterized⁶ as the antigen reacting with antibody produced in the human as a result of infectious mononucleosis. The use of beef rather than sheep cell extractives rules out the Forssman antigen, which complicates the picture in red cell agglutination tests with antibody from infectious mononucleosis patients. The surface blood cell antigens have been described⁷ as chemically similar to the heterophile antigens. Therefore, methods of extraction and assay for the one might prove valid for the other. Although the main objective was to test the value of the extraction method, it was hoped also that an assay using complement fixation technique might be derived which would detect the presence of heterophile antibodies earlier in the course of disease than is now possible.

BARNARD⁸ has confirmed the specificity of the hapten extracted from human red cells. This hapten was assayed by him, as well as in this laboratory, through complement fixation techniques. The sample studied by BARNARD was prepared by the writer using techniques reported elsewhere³. Beef cells were treated in an identical manner, i.e., one volume of fresh cells is precipitated by two volumes of 95% ethyl alcohol. This is allowed to stand over night at room temperature, then the precipitate is separated by filtration and added to two volumes of dichloromethane. This is mixed and kept at room temperature for 6 h or longer. The liquid is evaporated, leaving a lipid fraction.

The residue is dissolved in a proportion of 100 mg lipid per 1 ml alcohol. This is stable for several months at room temperature. For a complement fixation assay,

¹ B. B. CARTER, Amer. J. clin. Path. 17, 646 (1947).

² B. B. CARTER, J. Immunol. 61, 79 (1949).

³ B. B. CARTER, Exper. 12, 150 (1956).

⁴ C. HOWE and R. RUSTIGIAN, J. Immunol. 64, 505 (1950).

⁵ C. J. EHRENBURG, J. Lancet 75, 275 (1955). – E. MAYR, Geburtsh. Frauenheilk. 11, 239 (1951). – J. W. GOLDSMITH, Amer. J. Obst. Gyn. 59, 172 (1950).

⁶ J. TOMCSIK and H. SCHWARZWEISS, Proc. Soc. exp. Biol. 69, 562 (1948).

⁷ P. L. CARPENTER, Immunology and Serology (W. B. Saunders, Philadelphia 1956), p. 171.

⁸ R. L. BARNARD, 5th Int. Congr. Blood Transfusion (1955), p. 85.

⁶ G. GARDOS, Acta physiol. Acad. Sci. Hungar. 6, 191 (1954).

the extract is further diluted with alcohol to give a 1:100 dilution. Complement fixation techniques were set up, using both 100% and 50% end-points. The former technique was that described by KOLMER⁹ and the 50% end-point method was after KABAT and MAYER¹⁰. For the tests the beef cell antigen was diluted with 0.9% buffered saline to 1:1000, 1:2000, 1:4000, and 1:8000. These dilutions were tested for hemolytic and anti-complementary properties.

Heterophile antibody was provided by serums from patients suffering or convalescing from infectious mononucleosis. To test specificity, serums from individuals suffering from other diseases, as well as those from apparently normal individuals, were included. Titrations of amboceptor and complement were made according to standard techniques. For the 100% end-point, 0.2 ml serum stood for 10 min with 0.5 ml appropriate antigen dilution, 1 ml complement according to titer was added and, with suitable controls, the tests were kept at 4°C for 18 h. Then they were kept at 37°C for 10 min and to each test was added 1 ml sensitized 2% sheep red cells. A previous sensitization with an excess of amboceptor obviates possible competition between heterophile antibody and amboceptor for the sheep cells.

For the tests with a 50% end-point, 3, 6 and 12 units of complement were incubated for 4 h at 4°C with immune serum plus antigen, sensitized cells were added and results read after 15 min incubation at 37°C, on the basis of von KROGH's¹¹ equation: if a given serum permits 20% haemolysis under the conditions of the test, how much complement would be required to produce 50% haemolysis with the same serum?

35 serums from patients diagnosed as suffering or convalescing from infectious mononucleosis were tested in complement fixation reactions with the lipid fraction from beef red cells as antigen. These gave what appeared to be a specific fixation of complement. 15 supposedly normal serums were tested in the same manner and gave negative reactions. 15 serums from persons diagnosed as suffering from a variety of ills were tested also and gave negative results, with the exception of one serum from a person supposedly ill with viral hepatitis. This gave a weakly positive reaction with both the 100% and 50% end-point methods. It may be that here was a cross reaction or possibly the patient had infectious mononucleosis.

In 10 of the 35 infectious mononucleosis cases, it was possible to obtain serum before the convalescent period. Tentative diagnosis had been made on the appearance of the blood smear; the serum was still negative. An attempt was made to demonstrate heterophile antibodies in each serum before they were demonstrable by the PAUL-BUNNELL reaction¹². In this preliminary assay, the effort failed. Heterophile antibodies were not demonstrable by complement fixation prior to positive reactions with the agglutinating tests. There seemed to be a parallelism in the results with the two techniques. This was a disappointment because absolute diagnosis of infectious mononucleosis at present is a matter of

hindsight. The complement fixation reaction as investigated here did not seem to alleviate this situation.

Nevertheless, it would seem that the production of heterophile antigen from beef red cells and its employment in a complement fixation test is both practicable and profitable using the methods outlined above. Since the techniques are identical with those used to obtain fractions from Rh positive human red cells, which appear to react specifically with anti-Rh serums, it would seem to be an easily applied method for obtaining lipid haptens from various biological materials. This inference could have extensive therapeutic as well as experimental implications.

BETTINA B. CARTER

Western Michigan University, Kalamazoo, Michigan, April 23, 1957.

Zusammenfassung

35 Sera von Patienten mit infektiöser Mononukleose (10 Kranke, 25 Rekonvaleszenten), 15 Sera von Patienten mit anderen Krankheiten und 15 normale Sera wurden im Komplementbindungstest geprüft, wobei als Antigen ein Lipoidextrakt von Rinder-Erythrozyten verwendet wurde. Sera von Rekonvaleszenten nach infektiöser Mononukleose zeigten eine spezifische Komplementbindung, während bei Patienten vor der Rekonvaleszenz die heterophilen Antikörper im Komplementbindungstest nur dann nachgewiesen werden konnten, wenn die Paul-Bunnellsche Reaktion auch positiv ausfiel.

Activation de la phosphatase acide par l'acide β -indolylacétique et ses répercussions sur la phosphorolyse de l'amidon

Nous avons récemment montré que l'acide β -indolylacétique (hétéroauxine) aux concentrations phytopathologiques de 10^{-3} – 10^{-4} M stimule l'activité phosphatasique acide d'extraits de pomme de terre et de racine de maïs¹. Nous avons retrouvé une telle interaction dans les tissus hypertrophiés des Euphorbes castrées par *Uromyces*².

Il nous restait donc à confirmer ces premières données avec de la phosphatase acide purifiée. Les essais rapportés ci-dessous ont été réalisés avec de la phosphatase acide de germe de blé (produit Light).

La méthode et les procédés d'analyse ont été décrits ailleurs¹. Nous avons simplement remplacé l'extrait phosphatasique brut par 0,5 ml d'une solution à $1/4000$ de phosphatase pure dans l'eau distillée. L'activité glycérophosphatasique a été mesurée à 25 et 37°, en tampon acétate à pH 5,1 (optimum phosphatases type II³).

La Figure résume nos résultats obtenus en présence de différentes concentrations d'acide β -indolylacétique (ABIA).

Ces essais ont donc permis de confirmer la nette activation de la fonction hydrolysante de la phosphatase acide végétale par l'hétéroauxine. Nous pouvons en effet

⁹ J. A. KOLMER and F. BOERNER, *Approved Laboratory Technic* (D. Appleton-Century Co., New York 1945), p. 674.

¹⁰ E. A. KABAT and M. M. MAYER, *Experimental Immunochimistry* (Charles C. Thomas, Springfield, Ill., 1948), p. 176.

¹¹ A. B. WADSWORTH, *Standard Methods* (Williams and Wilkins, Baltimore 1939), p. 238.

¹² J. R. PAUL and W. W. BUNNELL, *Amer. J. med. Sci.* **183**, 90 (1932).

¹ G. TURIAN, *Biochim. biophys. Acta* **21**, 388 (1956).

² G. TURIAN, *Phytopath. Z.* **28**, 275 (1957).

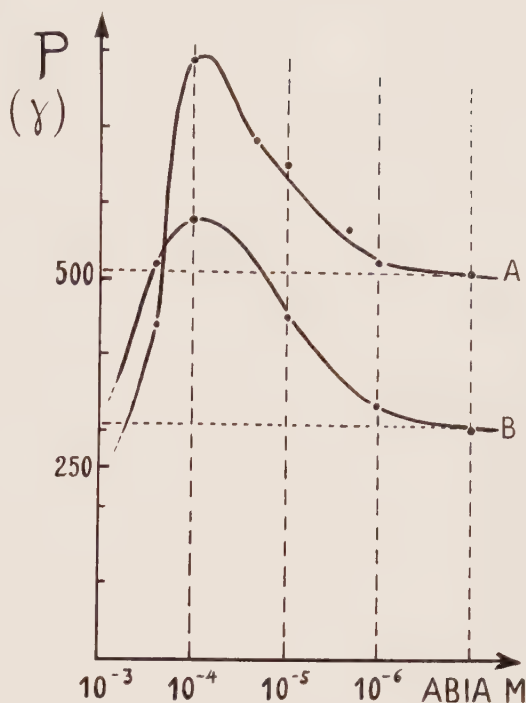
³ J. ROCHE et J. COURTOIS, *Exposés ann. Biochim. méd.* **4**, 219 (1944).

calculer qu'en 30 min, à 25°, 36% du glycérophosphate de Na initial (10 mg) est hydrolysé par 1 mg de phosphatase en présence de 10^{-4} M d'ABIA et 19% seulement dans les témoins placés dans les mêmes conditions.

La concentration activante optimale d'ABIA est plus faible pour la phosphatase purifiée (10^{-4} M) que pour les extraits phosphatasiques bruts (10^{-3} M). Cette différence relève peut-être d'une réduction du taux effectif d'ABIA par l'activité auxine-oxydasique de ces extraits bruts.

WILDMAN et BONNER⁴ ont signalé la simultanéité de la présence de l'ABIA et de l'activité phosphatasique lors de leur purification de protéines foliaires d'épinard. De même, nous pouvions supposer que l'activité relativement élevée des préparations phosphatasiques témoins (Figure) dépendait de traces d'ABIA présentes dans l'échantillon de phosphatase «pure». Nos premiers essais de chromatographie sur papier, avec de la phosphatase (5 mg) soumise à une hydrolyse alcaline (NaOH *N*/100) et extraite au chloroforme, ne nous ont toutefois pas permis de déceler l'ABIA (solvent: butanol-éthanol-eau; révélateur: acide perchlorique-FeCl₃). Seule la méthode de détection biologique, plus sensible, permettra d'éclaircir ce point.

Notre confirmation d'une interaction ABIA-phosphatase acide végétale nous a conduit à réexaminer la question d'une intervention des auxines dans le métabolisme de synthèse et de dégradation de l'amidon⁵. L'amylase exocellulaire ne semble en effet pas être influencée par la présence de l'ABIA⁶. Par contre, une médiation phosphorée lors des transformations endocellulaires de l'amidon a été avancée par divers auteurs⁷. Nous avons alors



Activité glycérophosphatase (à pH 5,1) de la phosphatase acide de germe de blé, en présence de diverses concentrations d'acide β -indolylacétique (ABIA): γ de P libérés par mg de phosphatase, en 30 min à 37° (A) et à 25° (B). Lignes horizontales en pointillé: activité des témoins.

	1	2	3	4	5	6	7	8	9
Amidon, 0,5 ml sol. 0,5% en tampon phosphate pH 5,4	+	+	+	+	+	+	+	+	+
«Phosphorylase», 0,2 ml	0	+	+	0	0	+	+	+	+
Phosphatase acide, 0,5 ml sol. $\frac{1}{2000}$	0	0	0	+	+	+	+	+	+
H ₂ O distillée (ml)	1,0	0,8	0,8	0,5	0,5	0,3	0,3	0,3	0,3
H ₂ O alcoolisée à 20% (ml)	1,0	1,0	0	1,0	0	1,0	0,75	0,50	0
ABIA 10 ⁻³ M, en sol. H ₂ O alcoolisée à 20% (ml)	0	0	1,0	0	1,0	0	0,25	0,50	1,0
Résultats: (estimation culot bleu noir à bleu violacé)	++++	+++	++	++(+)	++	+(+)	+	0	0

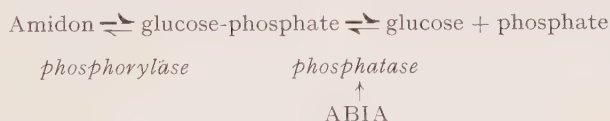
Volume réactif total = 2,5 ml. Concentrations d'ABIA = 10^{-4} M, $2 \cdot 10^{-4}$ M et $4 \cdot 10^{-4}$ M.

pensé que l'accélération de la dégradation de l'amidon en présence d'ABIA pourrait résulter de l'activation hormonale de la fonction hydrolysante de la phosphatase selon schéma plus bas). L'expérience suivante a permis de confirmer cette représentation.

Une série de petits tubes à essais contenant chacun 0,5 ml d'amidon soluble à 0,5% (sol. tampon pH 5,4), en présence ou en absence d'un extrait de germe de pomme de terre (source de phosphorylase), de phosphatase acide et d'ABIA, est incubée à 37° pendant 36 h. La

durée d'incubation précédant la révélation de l'amidon résiduel par le réactif de Lugol ($I_2 + IK$) a été fixée à la suite de divers essais préliminaires qui nous ont permis de constater qu'elle fournit la meilleure réaction différentielle, c'est-à-dire la disparition complète de l'amidon dans le mélange le plus actif (Tableau).

Il ressort de ce tableau que la dégradation de l'amidon est la plus complète en présence du mélange «phosphorylase»-phosphatase acide-ABIA. On peut donc admettre que l'ABIA contrôle le métabolisme endocellulaire de l'amidon grâce à son rôle de régulateur de l'activité phosphatasique acide dans les tissus végétaux, selon le schéma suivant :



⁴ S. G. WILDMAN et J. BONNER, Arch. Biochem. 14, 381 (1947).

⁵ M. K. BRAKKE et L. G. NICKELL, Arch. Biochem. Biophys. 32, 8 (1951). — S. C. BAUSOR, Bot. Gaz. 104, 115 (1942).

⁶ P. E. PILET et G. TURIAN, Bull. Soc. vaud. Sci. nat. 65, 403 (1953).

7 H. WANNER, Ber. schweiz. bot. Ges. 62, 205 (1952). — F. M. VERRI, Bol. Soc. Bot. Ital. 8, 125 (1953). — G. L. ROBERTSON, J. Ecol. 43, 115 (1955). — R. FUJII, Bot. Mag. Tokyo 69, 34 (1956).

Nous remercions M. le Prof. F. CHODAT de ses précieux conseils. Ces recherches sont subventionnées par le Fonds national suisse de la Recherche scientifique.

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Institut de Botanique générale, Université de Genève, le 21 mai 1957.

Summary

IAA-induced activation of purified acid phosphatase is reported and shown to determine an acceleration of phosphorolysis of starch.

Some Relationships Between the Pancreatic β -Cells and the Metabolism of the Epiphyseal Cartilage

I. Cartilage ATP Concentration of Young Alloxan Diabetic Rats

DE MOOR¹ and MARTINETTI, and ANDREANI² demonstrated that insulin deficiency provoked by alloxan diabetes produces, besides other biochemical and physiological alterations, a diminution of the body weight and of skeletal growth.

As the epiphyseal cartilage participates to the growth of the bone in length, and as in enchondral ossification the participation of the energetic phosphoric bonds is considerable (*i.e.* ATP, see CARTIER³), the author wishes to study whether the diminished growth observed in alloxan diabetic rats is accompanied in epiphyseal cartilage by a reduced concentration of ATP.

Albino male rats, Italicus strain, 50 days old, were divided into two groups. In the first group, after 12 h fasting, alloxan diabetes was provoked (Merck alloxan 200 mg/kg intraperitoneally); the second, which was the control group, was not treated. Ten days after alloxan treatment, and after 12 h fasting, blood sugar was measured according to NELSON⁴, and in the diabetic rats (blood sugar 2.8–4.9 g/1000 cm³), analogously to those of the control group, body weight and blood sugar were controlled every 5th day for 20 days. Thirty days after the alloxan injection, having controlled body weight and blood sugar, all diabetic and normal rats, 12 h fasted, were anesthetized with Nembutal (5 mg/100 g body weight) and then killed by decapitation. The anterior and posterior limbs were removed as rapidly as possible, and placed in a beaker containing acetone and dry ice: the epiphyseal cartilages were weighed, homogenized with quartz sand and CCl₃COOH 10% at 0°C. After centrifugation, to the supernatant fluid, neutralized to pH 8.2, barium acetate was added. In the precipitate (barium insoluble fraction) ATP was measured after LE PAGE⁵. P was dosed according to the BEREMBLUM and CHAIN method⁶. It is necessary to emphasize the

importance in such experiments of choosing rats of the same age: as a matter of fact, in a preliminary research, I observed that the ATP concentration of the epiphyseal cartilage shows, even for difference in their ages of 15–20 days, statistically significant variations. The decrease with age in ATP concentration in ossifiable cartilage is to be attributed to a progressive decrease during the growth of the intensity of the osteogenic power. Analogous observations, relative to the enchondral ossification, were made by ZAMBOTTI⁷ on the behaviour of the cocarboxylase and β -glucuronidase, and by LORENZI on the behaviour of concentration of pyruvic acid⁸.

The results obtained can be summarized as follows:

(1) The alloxan diabetic rats showed a remarkable diminution of the body weight with a check of growth (mean body weights at the start = 60 g; 30 days after alloxan treatment = 30 g. In the normal rats, the body weight increased from 60 g to 160 g in 30 days).

(2) The ATP concentration in epiphyseal cartilage of alloxan diabetic rats decreased by about 40% relative to normal rats (epiphyseal cartilage of normal rats = 20 mg P/ATP/100 g of wet tissue; epiphyseal cartilage of diabetic rats = 12 mg P/ATP/100 g of wet weight).

Such results clearly show that in alloxan diabetes the inhibition of skeletal growth is accompanied with a diminution of the ATP concentration in the epiphyseal cartilage.

To control whether the decreased ATP concentration in epiphyseal cartilage of alloxan diabetic rats can be attributed exclusively to the insulin deficiency, a group of albino male rats (of age and strain identical to those used in previous experiments) were treated with insulin (Zn-insulin protamine 4 U.I./kg daily subcutaneously), ten days after alloxan treatment (blood glucose 2.8–4.8 g/1000 cm³). The results showed that in insulin-treated diabetic rats, hyperglycemia was much less intense than in untreated diabetic rats. In addition, 30 days after alloxan injection, the body weight was seen to be the same as that of the normal rats (*i.e.* 160 g) as was also the ATP concentration in epiphyseal cartilage (mg 20 P/ATP/100 g wet weight).

Therefore, in alloxan diabetic rats, both the decreased ATP concentration and the diminished osteogenic power of the epiphyseal cartilage, are to be referred exclusively to the insulin deficiency.

It may also be suggested that insulin plays an important role in regulating enchondral ossification, especially through its marked control of the multienzyme system which pertains in ATP metabolism and consequently in all biochemical reactions which are dependent on ATP (*i.e.* biosynthesis of glycogen from glucose; biosynthesis of cocarboxylase and chondroitin sulphate, etc.). However, this hypothesis requires further evidence.

These experimental results, besides explaining one of the mechanisms by which insulin influences enchondral ossification, are in good agreement with CARTIER's *in vitro* findings: the primary importance of ATP for normal behaviour in the osteogenic process³.

E. BARBIERI

Institute of Clinical Orthopedics G. Gaslini, University of Genoa (Italy), April 23, 1957.

¹ P. DE MOOR, *Exper. Diab.* (Ed. Masson, Paris 1953).

² R. MARTINETTI and G. ANDREANI, *Boll. Soc. ital. Biol. sper.* 23, 582 (1947).

³ P. CARTIER, *Exposés Ann. Biochim. Méd.* 14, 73 (1952).

⁴ N. NELSON, *J. biol. Chem.* 153, 375 (1944).

⁵ G. A. LE PAGE, *Manometric Techniques and Tissue Metabolism* (Burgess Publishing Co., ed., 1949), p. 185.

⁶ I. BEREMBLUM and E. CHAIN, *Biochem. J.* 32, 295 (1938).

⁷ V. ZAMBOTTI, *Il Farmaco*, ed. scient. 10, 1017 (1955). – V. ZAMBOTTI and G. L. LORENZI, *Boll. Soc. ital. Biol. sper.* 29, 1953).

⁸ G. L. LORENZI, *Minerva Ortopedica* 7, April 1956.

Riassunto

È stato studiato il contenuto di ATP nella cartilagine epifisaria di giovani ratti diabetici per allossana. Il deficit insulinico da diabete allossanico provoca, oltre ad arresto dell'accrescimento scheletrico, una diminuzione della concentrazione di ATP nella cartilagine di coniugazione. Il significato biologico di questo risultato è messo in relazione col meccanismo d'azione dell'insulina e con l'importanza dell'ATP nel processo di ossificazione endocranale.

Some Relationships Between the Pancreatic β -Cells and the Metabolism of the Epiphyseal Cartilage

II. Cartilage Cocarboxylase Activity of Young Alloxan Diabetic Rats

ZAMBOTTI¹ first discovered the presence of cocarboxylase in the epiphyseal cartilage. It was also established by ZAMBOTTI that this enzyme is strictly related to the intensity of the osteogenic process: in fact he showed that the cocarboxylase activity in the epiphyseal cartilage of young rabbits is inversely proportional to their age. I have reached identical conclusions in my researches in which the participation of cocarboxylase in various phases of evolution of the normal fracture callus² and also the behaviour of the cartilage cocarboxylase during growth in conditions (avitaminosis C) of reduced osteogenic power were studied³. These relationships, shown first by ZAMBOTTI, explain the histological and histochemical alterations of the epiphyseal cartilage demonstrated by ROASENDA and CAMURATTI³ in deficiency of a particular vitamin which has the cocarboxylase as its active form, i.e. vitamin B₁.

I have studied the behaviour of the cocarboxylase in the epiphyseal cartilage of alloxan diabetic rats for the following reasons:

(1) Insulin deficiency is accompanied by an arrest of growth and alterations of osteogenic power of ossifiable cartilage⁴.

(2) There is a strict relationship between the intensity of the osteogenic process and the amount of cocarboxylase activity⁵.

(3) The diminution of the osteogenic power provoked by insulin deficiency is accompanied by a reduced concentration of ATP in the epiphyseal cartilage⁶ in the alloxan diabetic rats. It is known that ATP is in large measure synthesized by means of the oxidation of two α -ketoacids (pyruvic and α -ketoglutaric)⁷ for the oxidative utilization of which the cocarboxylase is essential.

(4) There is also a strict relationship between ATP and cocarboxylase regarding their metabolism. In fact, the biosynthesis of cocarboxylase from vitamin B₁

comes about by means of the reaction ($B_1 + ATP \rightarrow$ cocarboxylase + AMP)⁸ catalyzed by the thiaminkinase, an endoergonic reaction which, since it receives the energy liberated by the demolition of ATP, should occur with minor intensity when the concentration of ATP is diminished.

Albino male rats, of Italicus strain, 50 days old, were divided into two groups. In one of these groups alloxan diabetes was provoked (200 mg of Merck alloxan, intraperitoneally). The control group was not treated. Ten days after the alloxan treatment, the body weight and blood sugar were controlled and then the diabetic rats (blood glucose after 12 h of fasting = 2.8–4.9 g/1000 cm³) were controlled for another 20 days, with periodical examinations (control of body weight and of blood sugar every 5th day). 30 days after the alloxan treatment all the rats, diabetic and normal were killed, their body weight and blood sugar being controlled. The limbs were removed and placed in ice water. The cocarboxylase of the epiphyseal cartilages was extracted and measured according to the OCHOA and PETER's method⁹, as modified by SILIPRANDI¹⁰.

In the epiphyseal cartilages of the diabetic rats (which presented an arrest of skeletal growth identical to that previously described⁶) the cocarboxylase activity was reduced in respect to that in the control group (normal rats = γ 6.2/g wet weight; alloxan diabetic rats = γ 3.45/g wet weight). Results identical to those obtained in the normal rats were also demonstrated in diabetic rats treated with insulin (daily subcutaneous injection of Zn-insulin protamine, 4 U.I./kg), from the 10th to the 13th day after the alloxan injection. It is therefore concluded that the cocarboxylase content decrease in the epiphyseal cartilage accompanying the arrest of the skeletal growth can be attributed in the alloxan diabetic rats to the insulin deficiency.

Coordinating my results on ATP and cocarboxylase with those obtained by other authors¹¹, it seems logical to deduce that a deficient production of insulin induces an arrest of osteogenesis provoking a reduced synthesis of ATP by damaging those reactions in which cocarboxylase is interested. On the other hand, the analogous behaviour of ATP and cocarboxylase in insulin deficiency leads us to suppose that in epiphyseal cartilage the metabolism of these two substance is interdependent: the insulin by means of the energy furnished by the ATP supports the transformation of thiamin into diphosphothiamin; the latter, in its turn, participates in the formation of ATP by means of the pyruvate and α -ketoglutarate oxidative decarboxylation. This hypothesis on the mechanism of the participation of the insulin in the osteogenic process by means of ATP and cocarboxylase is very probable, other than for my present results (osteogenesis normalization in the diabetic rats simultaneously with normalization of the amount of ATP and of cocarboxylase in the epiphyseal cartilage, after insulin treatment) also for my other experimental data: alloxan diabetes induces in the epiphyseal cartilage a diminished pyruvate and α -ketoglutarate oxidation; this process which is in strict relationship with the ATP synthesis⁷, is also normalized by insulin treatment.

¹ V. ZAMBOTTI, *Il Farmaco*, ed. scient. 10, 1017 (1955).

² E. BARBIERI, unpublished data.

³ F. ROASENDA and C. CAMURATTI, *Minerva Ortopedica* 3, 170 (1952).

⁴ P. DE MOOR, *Exper. Diab.* (Ed. Masson, Paris 1953). – R. MARTINETTI and G. ANDREANI, *Boll. Soc. ital. Biol. sper.* 23, 582 (1947).

⁵ V. ZAMBOTTI, *Il Farmaco*, ed. scient. 10, 1017 (1955). – E. BARBIERI, unpublished data.

⁶ E. BARBIERI, *Exper.*, previous communication.

⁷ H. A. KREBS, *Exposés Annuels de Biochimie Médicale* 15, 11 (1952).

⁸ F. LEUTHARDT and H. NIELSON, *Helv. chim. Acta* 35, 1196 (1952).

⁹ S. OCHOA and R. A. PETERS, *Biochem. J.* 32, 1501 (1938).

¹⁰ D. SILIPRANDI and N. SILIPRANDI, *Acta Vitaminol.* 5, 3 (1951).

¹¹ R. MARTINETTI and G. ANDREANI, *Boll. Soc. ital. Biol. sper.* 23, 582 (1947). – E. BARBIERI, *Exper.*, previous communication.

These results indirectly confirm the significance of the ATP, demonstrated by CARTIER¹², and of the co-carboxylase, demonstrated by ZAMBOTTI¹, for a normal behaviour in the osteogenic process.

E. BARBIERI

Institute of Clinical Orthopedics G. Gaslini, University of Genoa (Italy), April 23, 1957.

Riassunto

Studiando il meccanismo biochimico con cui la insulina controlla il processo di osteogenesi, si è stabilito che in condizioni di deficit insulinico (diabete allossanico) esiste nel ratto accanto ad un arresto dell'accrescimento scheletrico, una notevole riduzione dell'attività cocarbossilica nella cartilagine epifisaria. Si conclude che nella cartilagine di coniugazione ATP e cocarbossilasi sono strettamente interdipendenti per quanto riguarda la loro biosintesi; inoltre che il loro normale metabolismo è indispensabile al normale svolgersi del processo di osteogenesi.

¹² P. CARTIER, *Exposés Ann. Biochim. Méd.* 14, 73 (1952).

Influence of Post Irradiation Administration of Methionine on the Excretion of Creatine, Creatinine and N-Methyl Nicotinamide in Urine of Rats

Earlier observations from this laboratory have demonstrated a precipitous fall in the levels of free-methionine and choline in rat livers¹ and an enhanced excretion of methylated end-products such as creatine, creatinine and N'-methyl-nicotinamide in urine of rats given 600 r total body X-ray irradiation². This implied that radiation exposure probably disrupted the methyl economy and suggested that there may be a cause-and-effect relationship between radiolability of methionine³ and the levels of the metabolites studied. These considerations prompted us to study how far replacement therapy of methionine would have beneficial effects in modifying the reported biochemical lesions

of radiation exposures. In this communication, the influence of post-irradiation administration of methionine on the excretion of creatine, creatinine, N'-methyl-nicotinamide and nitrogen in urine is reported. The influence of giving methionine half an hour before irradiation has also been investigated.

Experimental.—1½ to 2 month old wister rats, bred in our colony, were used in this investigation. The animals were divided into 5 groups of 6 rats each, as follows. The first group served as controls and the animals did not receive any treatment at all. The second group of animals were not irradiated but received injection of methionine at a dose rate of 5 mg/kg of body weight. The animals in the 3rd group were irradiated with 600 r X-rays. In the 4th group, animals were irradiated and received methionine ½ h after irradiation. The last group was also irradiated but received methionine before irradiation.

All animals, control as well as irradiated, were fasted for 24 h during the collection of urine. Drinking water was, however, made available to them *ad libitum*. Animals were housed in metabolic cages in pairs to facilitate the collection of urine.

For the estimation of creatine and creatinine, the urine samples were first treated with iodine-iodide reagent as described by TAUSSKY⁴ to remove the interfering substances, followed by extraction of the excess of iodine with chloroform. Urine samples were then adjusted to pH 2.5 and taken for the estimation of creatine and creatinine. The conversion of creatine to creatinine was carried out by autoclaving the urine samples (pH 2.5) as recommended by CLARK *et al.*⁵. Preformed creatine and total creatinine was then estimated colorimetrically⁴ by adding 1 ml of 0.04 N picric acid and 1 ml of 0.75 N NaOH. After 20 min of stabilization, the colour intensity was measured in Klett Summerson colorimeter with green filter.

For the estimation of N'-methyl-nicotinamide, the urine samples were treated with acetone and 6 N NaOH, followed by addition of 6 N HCl to produce stable fluorescent condensation product as described in detail by HUFF and PERLZWEIG⁶. The fluorescence was measured in Pfattz and Baur instrument with the combination of green and yellow filters.

Nitrogen in urine samples was estimated colorimetrically by nesslerization procedure as outlined by UMBREIT *et al.*⁷.

¹ U. S. KUMTA, S. U. GURNANI, and M. B. SAHASRABUDHE, *Current Science* 24, 362 (1955).

² U. S. KUMTA, S. U. GURNANI, and M. B. SAHASRABUDHE, *in press*.

³ U. S. KUMTA, S. U. GURNANI, and M. B. SAHASRABUDHE, *J. Sci. Ind. Res. (India)* 16-C, 25, 1957. — U. S. KUMTA, S. U. GURNANI, and M. B. SAHASRABUDHE, *J. Sci. Ind. Res. (India)* 16-C, 111, 1957.

⁴ H. H. TAUSSKY, *J. biol. Chem.* 208, 853 (1954).

⁵ L. C. CLARK and H. L. THOMPSON, *Anal. Chem.* 21, 1218 (1949).

⁶ J. W. HUFF and W. A. PERLZWEIG, *J. biol. Chem.* 167, 157 (1947).

⁷ W. W. UMBREIT, R. H. BURRIS, and J. F. STAUFFER, *Manometric techniques and related methods for the study of tissue metabolism* (Burgess Publishing Co., Minneapolis 1949).

Influence of administration of methionine (5 mg/kg of body weight) on the excretion levels of creatine, creatinine, N'-methyl nicotinamide and nitrogen in irradiated animals.

No. of rats	Group	Nitrogen mg/day/rat	Creatine mg/day/rat	Creatinine mg/day/rat	N'MeNA mg/day/rat
4	Control	56.5 ± 0.5	0.54 ± 0.089	1.96 ± 0.28	0.236 ± 0.004
6	Control + Methionine	52.6 ± 1.52	0.57 ± 0.07	1.8 ± 0.037	0.198 ± 0.003
6	Irradiated	92.8 ± 4.3	1.19 ± 0.11	3.87 ± 0.24	0.425 ± 0.009
6	Irradiated + Methionine given <i>before</i> irradiation	85.63 ± 4.8	1.3 ± 0.2	3.42 ± 0.38	0.215 ± 0.005
6	Irradiated + Methionine given <i>after</i> irradiation	85.8 ± 3.39	0.463 ± 0.001	3.08 ± 0.12	0.212 ± 0.006

Standard errors have been calculated using the formula $(\sum d^2/n(n-1))^{\frac{1}{2}}$.

Results and Discussion.—The results of these experiments are presented in the Table. It will be seen that administration of methionine half an hour after irradiation brought back the excretion levels of creatine and N'-methyl-nicotinamide to normality, but it does not appear to have any effect on the excretion of creatinine. This continued to be high as in the case of untreated irradiated animals.

Administration of methionine half an hour before irradiation does not seem to control the increased excretion of creatine and creatinine. The only effect it had was to restore the N'MeNA levels to normality. Similarly, the excretion of nitrogen continued to be high in both the groups, thereby showing that the increased catabolic activity is not reversed by methionine.

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Biology Division, Atomic Energy Establishment, Indian
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Zusammenfassung

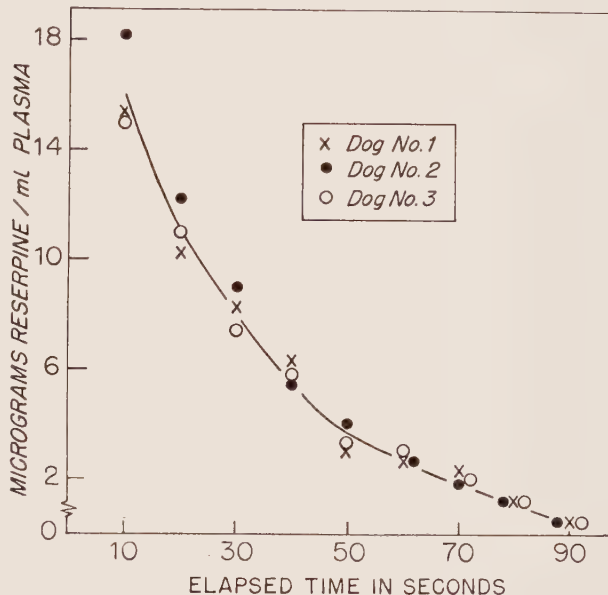
Die Möglichkeit wurde geprüft, durch Zugabe von Methionin die Kreatin-, Kreatinin-, N'-Methylnicotinamid- und Stickstoffmengen nach Bestrahlung wieder herzustellen. Bei Methioninzugabe eine halbe Stunde nach Bestrahlung kehrte die Kreatin- und N-Methylnicotinamidmenge im Urin zur Norm zurück; wurde aber Methionin vor der Bestrahlung verabreicht, so blieben die Ausscheidungsmengen von Kreatin, Kreatinin und Stickstoff hoch.

Plasma Disappearance and Urinary Excretion of Reserpine (Serpasil®) in the Unanesthetized Dog*

Recent studies in the rat¹ and rabbit² indicate that the sedative effect of reserpine may not be related in time to the concentration of the drug in arterial plasma, but rather, to changes in brain 5-hydroxytryptamine concentration. Since *in vitro* and *in vivo* observations³ suggest that there is a distinct species difference in the way reserpine is metabolized, the distribution of reserpine in arterial plasma of the dog, as a function of time, was investigated in an attempt to explain the particular sensitivity of this animal to small doses of the compound. The study was also to serve as a preliminary model for investigation of the renal handling of the drug.

A microcolorimetric method, based upon a general reaction for organic bases⁴, was developed and utilized for the analysis of reserpine⁵. 6 experiments were performed on 3 unanesthetized female mongrel dogs weighing between 12.0 and 14.3 kg. They were trained

and allowed free access to Purina Dog Chow and tap water. 25 ml of tap water per kg of body weight were given by mouth through a stomach tube one-half hour prior to the beginning of each experiment to insure adequate hydration and urine flow. Each animal was loosely restrained to a dog board in the supine position and an indwelling catheter placed in the bladder. The femoral artery was exposed under local anesthesia (distilled water) and a polyethylene catheter inserted into the proximal end. Control samples of urine and blood were taken. 15 min were allowed for surgical recovery. Reserpine, 1.5 mg/kg body weight, was then injected into the left external jugular vein over a 3 s interval, and arterial blood samples were taken at 10 s intervals for the next 2 min. The midpoint of the injection time was designated as zero time. Catheterized urine samples were collected at 30 and 60 min, and 24 and 48 h samples were cage-collected.



Arterial plasma disappearance of reserpine in the unanesthetized dog. The points plotted for each dog represent the averages of two experiments, and the resultant curve is a visual approximation. The broken line on the ordinate indicates the lower limit of sensitivity of the analytical method.

The disappearance of reserpine from arterial plasma is shown in the Figure. After intravenous administration, there was a prompt appearance of reserpine in arterial plasma with an average peak concentration of 16 µg/ml occurring during the first 10 s sample. Reserpine concentration then rapidly diminished to levels of less than 1.5 µg/ml of arterial plasma within 90 s.

Recovery of reserpine from urine is shown in the Table. Less than 2% of an injected dose of reserpine was recovered from urine within 24 h, and negligible amounts were found in the course of the next 48 h.

Sedation became evident at approximately 2–3 h after drug injection and persisted for 24–28 h. Tarry stools (markedly guaiac positive) were observed in all animals 3–6 h after injection. Stools were negative for occult blood within 24–48 h after injection. All dogs recovered fully in the course of one week.

The findings of a rapid disappearance from arterial plasma and low urinary excretion of reserpine are in general accord with observations of SHEPPARD *et al.*¹ in the rat using radioactive tracer doses of reserpine. HESS *et al.*², using 5 mg/kg of body weight in the rabbit,

* A grant for this study and the reserpine (serpasil®) used in these experiments, were generously given by CIBA Pharmaceutical Products, Inc., Summit, New Jersey.

¹ H. SHEPPARD, R. C. LUCAS, and W. H. TSIEN, Arch. int. Pharmacodyn. 103, 256 (1955).

² S. M. HESS, P. A. SHORE, and B. B. BRODIE, J. Pharmacol. 118, 84 (1956).

³ H. SHEPPARD, R. C. LUCAS, and W. H. TSIEN, Arch. int. Pharmacodyn. 103, 256 (1955). — S. M. HESS, P. A. SHORE, and B. B. BRODIE, J. Pharmacol. 118, 84 (1956). — H. SHEPPARD and W. H. TSIEN, Proc. Soc. exp. Biol. Med. 90, 437 (1955).

⁴ B. B. BRODIE and S. UNDEFRIED, J. biol. Chem. 158, 705 (1945).

⁵ E. A. DE FELICE (to be published).

Recovery of reserpine from urine

Dog number	Cumulative percentage of injected dose recovered as reserpine*			
	30 min	60 min	24 h	48 h
1 12.0 kg	0.18	0.25	1.4	1.6
2 13.5 kg	0.12	0.40	1.9	2.0
3 14.3 kg	0.24	0.39	1.6	1.7

* Figures represent averages of two experiments performed on each animal.

found urinary excretion to be of a similar magnitude, but the disappearance from plasma was somewhat more delayed. Observations of SHEPPARD *et al.*¹, HESS *et al.*², NUMEROF *et al.*⁶, and GLAZKO *et al.*⁷ indicate that the rapid disappearance of reserpine from arterial plasma reflects a quick degradation of the compound and not extensive binding in tissues. In addition, HESS *et al.*² claim that the same duration of sedation, or of change in brain 5-hydroxytryptamine, is induced whether a small or a large dose of reserpine is administered. Therefore, it is unlikely that reserpine is acting through a degradation product.

Since the sedative effect of reserpine was most marked at a time when most of the drug had disappeared from plasma, the results of this paper are in accord with the hypothesis² that the central effect of sedation is not mediated through reserpine *per se*, but conceivably through 5-hydroxytryptamine changes in brain.

The disappearance of reserpine from arterial plasma does not appear to explain the marked sensitivity of the dog to small doses of the drug, but there is other evidence that this animal metabolizes reserpine differently than other species⁸.

The negligible urinary excretion of reserpine precludes further studies on the renal handling of the drug.

E. A. DE FELICE**

Department of Biological Sciences, New England College of Pharmacy Boston, Mass., May 21, 1957.

Résumé

La réserpine administrée par voie intraveineuse chez le chien à dose de 1,5 mg/kg de poids du corps disparaît rapidement du sang artériel. Les quantités de réserpine

⁶ P. NUMEROF, M. GORDON, and J. M. KELLY, *J. Pharmacol.* 115, 427 (1955).

⁷ A. J. GLAZKO, W. A. DILL, L. M. WOLF, and A. KAZENKO, *J. Pharmacol.* 118, 377 (1956).

⁸ H. SHEPPARD and W. H. TSIEN, *Proc. Soc. exp. Biol. Med.* 90, 437 (1955).

** Professor in Biological Sciences, New England College of Pharmacy, and CIBA Fellow in Pharmacology, Boston University, School of Medicine.

retrouvées sont de 1,5 µg/ml au bout de 90 s. Il n'y a pas de corrélation entre les quantités de réserpine contenues dans le sang et le début ou la durée de la sédation produite. Au bout de 48 h, moins de 2% de la dose injectée est retrouvée dans l'urine. Etant donné la rapidité avec laquelle la drogue disparaît dans du sang, on ne s'explique pas l'hypersensibilité du chien à son égard. L'excrétion rénale négligeable de la drogue chez le chien ne permet pas d'espérer que des études de clairance rénale éclaircissent le mécanisme d'action de la réserpine chez cet animal.

Short-term Oscillations in Sign of Phototaxis in the Eyed Hawk Caterpillar (*Smerinthus ocellata* L.)

In a previous communication¹, we have discussed the cyclic changes in phototaxis occurring in the later instars of the Eyed Hawk's larval life. The data then presented always referred to the average behaviour of a group of larvae of the same age, but in our experiments we have recorded the movements of each larva individually. Further analysis of these records has revealed an interesting fact. At all ages during the 3rd to 5th instars, periods in which the larva crawls towards the light alternate with periods in which it moves in the opposite direction (Fig. 1). This suggests that the sign of the caterpillars' phototaxis is subject to somewhat irregular short-term oscillations between positive and negative.

However, before we can accept this conclusion, another possible explanation must be considered. The occurrence of bouts of crawling in the 'wrong' direction in larvae showing an over-all preference for a given orientation, might be regarded merely as a sign that the phototaxis of these larvae is rather weak, the wrong movements being chance deviations from the 'intended' course. The following argument can be advanced against this hypothesis as the sole explanation of our findings. Figure 2 illustrates our scoring method (for further details, see the earlier paper). It will be seen that the sum of the two angles comprising our 0 directions is $\frac{2}{3}$ of the angle comprising either the + or the - directions. Hence, if the bouts of wrong scores were due to errors made by the larvae, we should expect the number of 0 scores to be greater than (or at least equal to) $\frac{2}{3}$ of the number of wrong scores in each test, as large chance deviations, leading the larva into the wrong sector, will be less frequent than smaller ones which take it only into the 0 sectors.

The facts do not confirm this expectation. In only 43 out of 133 larvae tested at varying ages in the 3rd to 5th instars, the difference expected on the hypothesis under consideration was actually found. In all others, the number of 0 scores was smaller than $\frac{2}{3}$ of the number of wrong scores. Moreover, the expected difference occurred more frequently among larvae giving a relatively small proportion of wrong scores than among

¹ L. DE RUITER and IJ. VAN DER HORN, *Nature* 179, 1027 (1957).

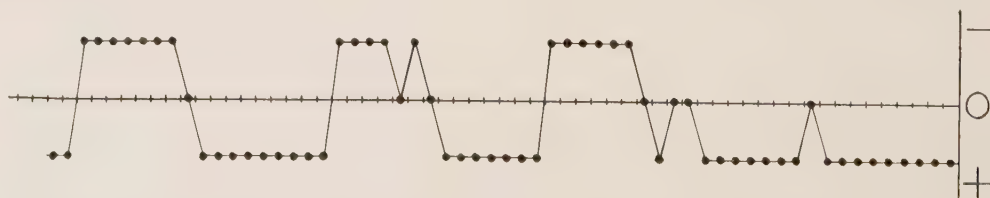


Fig. 1.—Third instar larva, observed at one-minute intervals. Alternation of movements towards and away from the light source (indicated by dots above and below the abscissa, respectively). Time scale: minutes.

those giving a large one. As a measure of this proportion in a given test (or, in other words, of the intensity of the larva's preference for one orientation) we may use the

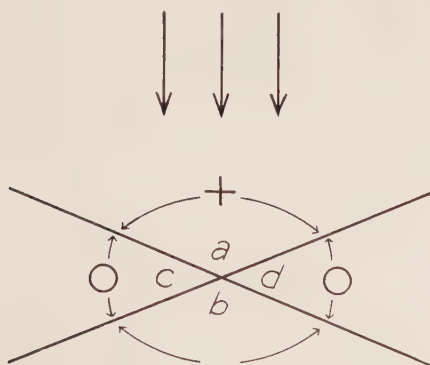


Fig. 2.—Scoring method. The arrows indicate the direction of the light. The signs +, —, and 0 indicate the scores given to larvae heading in directions within the respective sectors.

$$L a = L b = 135 ; L c = L d = 45^{\circ}.$$

absolute value of the difference D between the percentages of positive and negative scores obtained in that test. In this way we find:

D	Number of larvae	Percentage of these showing expected difference
0–20	21	9.5
21–40	16	12.5
41–60	15	20.0
61–80	20	45.0
81–100	61	44.3

This correlation between the value of $|D|$ and the occurrence of the expected difference seems inexplicable on the 'error hypothesis'.

In our opinion, our data suggest that in larvae with a strong preference, bouts of wrong movements may indeed be due in many cases (but not in all) to errors in orientation. However, for caterpillars giving low values of $|D|$ this explanation appears to hold in a small minority of all cases. Here a change in sign of the direction taken by the larva must far more often be due to a real change in the sign of the phototaxis itself.

The duration both of the positive and of the negative phases in the behaviour of a larva varies considerably, even within a single test. Therefore, more extensive experiments will be necessary to decide whether increase in length of the negative phases, or in their frequency, or in both, is the cause of the shift towards photonegativity to be seen in each instar¹. We are pursuing the work in this direction. In addition, we are attempting a further analysis of the oscillations in sign of phototaxis.

L. DE RUITER and IJ. VAN DER HORN

Zoological Laboratory of the University, Groningen (Netherlands), April 24, 1957.

Zusammenfassung

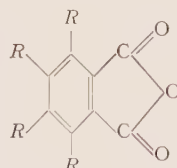
Raupen von *Smerinthus ocellata* L. im dritten bis fünften Larvenstadium zeigen im Phototaxisversuch unregelmässig periodische Umkehr der Kriechrichtung (Fig. 1). Aus der weiteren Analyse der experimentellen Daten ergibt sich, dass dieser Richtungswechsel in der Mehrzahl der Fälle durch einen tatsächlichen Umschlag des Richtungssinnes der Phototaxis verursacht wird.

PRO EXPERIMENTIS

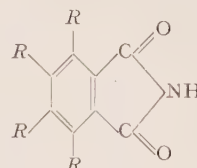
Sur une nouvelle méthode chromatographique pour la séparation de composés polycycliques aromatiques ou hétérocycliques

On sait que les techniques habituelles de chromatographie utilisent les différences existant entre les composants d'un mélange en ce qui concerne leur faculté de fixation physique sur un adsorbant approprié, constitué en général soit par des composés minéraux, soit par des substances organiques macromoléculaires.

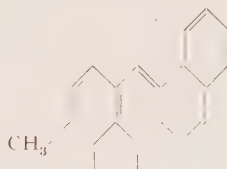
Nous proposons aujourd'hui l'utilisation d'une nouvelle technique chromatographique, basée sur l'utilisation, comme adsorbant, de composés tels que l'anhydride tétrachlorophthalique (I; $R = Cl$) et la tétrachlorophthalimide (II; $R = Cl$). Ces composés présentent en effet, à l'état dissous, une affinité prononcée vis-à-vis de certains types de donneurs d'électrons, tels que les hydrocarbures aromatiques polycycliques, les indoles, les carbazoles, etc., avec lesquels ils fournissent des combinaisons moléculaires de type 1:1 plus ou moins fortement colorées et plus ou moins stables¹. Cette affinité varie dans larges proportions avec la structure moléculaire des donneurs d'électrons, et existe également lorsque l'anhydride et l'imide tétrachlorophthaliques se trouvent à l'état solide. Dans ce dernier cas, on obtient des adsorbats tout comme dans le cas de la chromatographie ordinaire. Les anhydrides tétrabromo- (I; $R = Br$) et tétraiodophthalique (I; $R = I$), ainsi que les imides correspondantes (II; $R = Br$ ou I) peuvent également se comporter comme de bons adsorbants, les imides étant d'une façon générale moins satisfaisantes que les anhydrides. Si le long d'une colonne constituée avec de l'anhydride tétrachlorophthalique en poudre fine, on fait passer une solution dans l'éther de pétrole ou dans le cyclohexane d'un mélange de plusieurs corps ayant des affinités



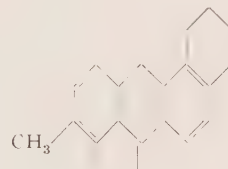
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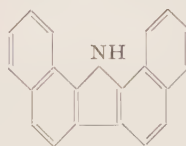
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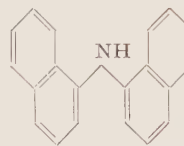
III



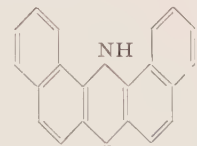
IV



V



VI



VII

¹ P. PFEIFFER, *Organische Molekülverbindungen* (F. Encke, Stuttgart 1927). — N. P. BUU-HOI et P. JACQUIGNON, *C. r. Acad. Sci.* 234, 1056 (1952); *Bull. Soc. chim. France* 1957, 488.

différentes vis-à-vis de cet anhydride, on obtient les phénomènes de résolution classiques de la chromatographie. Par exemple, un mélange de 20-méthylcholanthrène (III) et de son dérivé 1, 2, 3, 4, 11, 14-hexahydrogéné (IV) (on obtient un tel mélange dans l'hydrogénation de III)² peut ainsi être résolu en ses constituants, le 20-méthylcholanthrène se trouvant complètement adsorbé sur l'anhydride tétrachlorophthalique avec une coloration rouge vermillon, le dérivé hydrogéné restant dans la phase liquide à l'état de solution incolore. L'hydrocarbure adsorbé peut ensuite être élué de son support par passage à un pH alcalin, et repris par un solvant approprié. On peut séparer aisément de la même manière le pyrène (qui donne un adsorbat jaune orangé) de ses dérivés hydrogénés³ (1, 2, 6, 7-tétrahydropyrène, hexahydropyrènes, et décahydropyrènes), ou le 1, 2:7, 8-dibenzocarbazole (V) (qui donne un adsorbat de couleur rouge vif) de composés aussi voisins que l' α, α' -dinaphtylamine (VI), ou la 3, 4:5, 6-dibenzophénothiazine (VII), lesquelles restent en solution. Notre méthode est particulièrement utile pour la séparation et le dosage de faibles quantités d'hydrocarbures cancérogènes existant dans un milieu biologique complexe.

La présente technique chromatographique s'apparente aux méthodes analytiques de séparation qui sont basées sur la formation de composés d'inclusion⁴ avec les urées et les thiourées; elle se rapproche aussi des méthodes de résolution des racémiques, basées sur l'adsorption sélective d'un des énantiomorphes sur un support optiquement actif.

N. P. BUU-HOÏ et P. JACQUIGNON

Institut du Radium de l'Université de Paris, le 24 avril 1957.

Summary

A new chromatographic technique is described, which makes use of the affinity of anhydrides and imides of tetrahalogenophthalic acids towards aromatic and heterocyclic polycyclic compounds. This method has been successfully applied to the resolution of some problems of analytical organic chemistry.

² H. WIELAND et E. DANE, Z. physiol. Chem. 219, 240 (1933). — L. F. FIESER et H. HERSHBERG, J. Amer. chem. Soc. 60, 940 (1938).

³ J. W. COOK et L. C. HEWETT, J. chem. Soc. 1933, 401. — E. A. COULSON, J. chem. Soc. 1927, 1298.

⁴ Voir la littérature dans F. CRAMER, *Einschlussverbindungen* (Springer-Verlag, Berlin-Heidelberg 1954).

PRO EXPERIMENTIS

Méthode d'identification dans la flore intestinale d'une souche d'*Escherichia coli* implantée par voie buccale

L'inconvénient majeur des antibiotiques à large champ d'activité, plus spécialement lorsqu'on les administre par voie buccale, est de provoquer une raréfaction plus ou moins rapide de la flore microbienne saprophyte du tube digestif. Cette stérilisation relative de l'intestin prépare le terrain à divers microbes de superinfection, plus spécialement à certaines races pathogènes de staphylocoques, de streptocoques ou de colibacilles, de même qu'à certains champignons dont le plus régulièrement agressif est la *Candida albicans*.

Divers thérapeutes ont essayé, au cours des dernières années, de remédier à cet inconvénient par l'implantation, dans le tube digestif des malades ainsi menacés, de

souches d'*Escherichia coli*. De nombreux travaux ont été consacrés à ce problème. Ils se sont régulièrement heurtés à la difficulté de repérer à coup sûr les micro-organismes administrés et de les différencier de la flore intestinale préexistante. Les selles d'un individu normal renferment, en effet, une multitude de souches différentes d'*Escherichia coli*, dont le recours à la typisation sérologique de KAUFFMANN¹ permet seule l'identification. Il est possible, grâce à cette méthode, de reconnaître parmi ses congénères le colibacille d'implantation et de déceler son éventuelle persistance dans l'intestin du sujet traité.

Dans le but de vérifier l'efficacité de l'implantation, nous avons choisi un mutant, *Escherichia coli* «L» (*E. coli* «L»), présentant des caractères biochimiques nettement différents de ceux des colibacilles isolés chez une série de sujets dont nous avons au préalable étudié la flore intestinale saprophyte. La souche «L» est lactose⁻, xylose⁻ et galactose⁻; elle produit de l'indol à partir du tryptophane et attaque l'urée. De plus nous l'avons rendue résistante à 400 µg/ml de streptomycine, ce qui facilite son isolement, la plupart des germes normalement trouvés dans les selles étant sensibles à cet antibiotique.

La probabilité de l'existence naturelle dans l'intestin d'un microorganisme ayant les propriétés de *E. coli* «L» est négligeable. La présence de germes d'emblée résistants à de fortes concentrations de streptomycine est en revanche moins aléatoire. Il est dès lors indispensable d'avoir recours, lors des essais d'implantation microbienne, à un microorganisme qui ne soit pas identifiable uniquement par sa résistance.

Nos expériences ont porté sur quatre sujets en bonne santé qui ont été suivis, du point de vue bactériologique, durant cinq mois. Ils se sont alimentés comme d'habitude les trois premiers mois, puis ont été soumis à un régime protidique pendant une autre semaine, à un régime glucidique durant une autre semaine, après quoi ils ont repris leur alimentation habituelle. Au cours de cette période de cinq mois, 150 souches de colibacilles ont été isolées de leurs selles; aucune n'a présenté l'ensemble des caractères de *E. coli* «L».

Pour la recherche de *E. coli* «L», les selles ont étéensemencées sur plaques de gélose d'Endo contenant 400 µg/ml de streptomycine. Un gramme de selles, prélevé immédiatement après la défécation, est suspendu dans 10 ml de NaCl à 0,9 %, agité pendant 10 min, puis soumis à une série de dilutions. 0,1 ml de chacune de ces dilutions est ensemencé sur plaques d'Endo avec streptomycine. Tous les examens ont été pratiqués avant et après ingestion, à l'occasion d'un repas, d'une ampoule de bouillon contenant environ 10¹³ germes de la souche «L». Les colonies «L» apparaissent incolores, translucides, petites, tandis que la plupart des autres micro-organismes de la flore intestinale normale sont inhibés par la streptomycine ou présentent des caractères morphologiques qui permettent facilement leur différenciation d'avec *E. coli* «L». Les colonies «L» sont ensuite repiquées sur gélose ordinaire, puis réensemencées pour contrôle dans les différents milieux nécessaires à leur identification: lactose, xylose, galactose, tryptophane, urée².

Chez les quatre sujets soumis à l'expérience l'ingestion de *E. coli* «L» a été régulièrement suivie, après 18–20 h, de l'apparition de ce germe dans les fèces. Une étude

¹ F. KAUFFMANN, *Enterobacteriaceae* (Monksgaard, Copenhagen 1954).

² J. LEDERBERG, *Genetics* 32, 505 (1947).

quantitative, dont les résultats sont donnés dans le Tableau, a pu être réalisée chez deux d'entre eux.

Etude quantitative du nombre de colibacilles naturels et de colibacilles d'implantation (*E. coli* «L») décelables dans 1 g de matières fécales après ingestion d'une souche étrangère de microorganismes (*E. coli* «L»).

	Heures après ingestion de <i>E. coli</i> «L»	Nombre de <i>E. coli</i> par g de fèces	
		<i>E. coli</i> naturels	<i>E. coli</i> «L»
Sujet A	0	$2,0 \cdot 10^8$	—
Ingestion $6 \cdot 10^9$ <i>E. coli</i> «L»	24	$6,0 \cdot 10^7$	$2,0 \cdot 10^7$
	48	$1,7 \cdot 10^6$	$2,2 \cdot 10^7$
	70	$3,2 \cdot 10^6$	$2,0 \cdot 10^6$
Sujet B	0	$2,3 \cdot 10^8$	—
Ingestion $1 \cdot 10^9$ <i>E. coli</i> «L»	18	$2,3 \cdot 10^8$	$1,4 \cdot 10^8$
	22	$1,9 \cdot 10^8$	$1,5 \cdot 10^8$
	48	$2,2 \cdot 10^8$	$2,0 \cdot 10^8$

Les résultats obtenus montrent qu'un mutant d'*Escherichia coli*, présentant divers caractères qui permettent son identification biochimique et résistant à la streptomycine, apparaît dans les selles après avoir été ingéré. Des recherches sont actuellement en cours pour déterminer pendant combien de temps il peut persister dans le tube digestif. D'autres souches de colibacilles, identifiables grâce à certaines propriétés particulières, sont également à l'étude.

Y. POSTERNAK, R. REGAMEY,
P. RENTCHNICK et G. BICKEL

Clinique médicale universitaire et Laboratoire central de l'Hôpital cantonal de Genève, le 21 mai 1957.

Zusammenfassung

Die beschriebene Methode gestattet, das Überleben zu therapeutischen Zwecken oral eingeführter Stämme von *Escherichia coli* in der Darmflora weiter zu verfolgen.

PRO LABORATORIO

A Densitometric Adapter for Direct Plotting of Electrophoretic Curve from Paper Strips

Up to the present time there is perhaps no biochemical method of determination which, in such a short time, has spread so widely in clinical medicine as the paper electrophoresis of proteins¹.

In most cases the clinician must be satisfied with only the evaluation of the electrophoretic strip by estimation firstly because the quantitative measurement of single protein fractions by evaluation is very laborious and the special densitometric apparatuses required are expensive. Finally it is not possible nowadays to imagine a scientific publication in this branch without a documentation with an electrophoretogram².

In view of this situation, I developed the idea of constructing a simple adapter which would permit a densitometric evaluation of electrophoretic strip in a spectrophotometer. After nearly two years of different experiments and constructive improvements, I succe-

eded in constructing an adapter (Mod. IV) which is no longer an emergency solution but a perfect apparatus in which it is possible to register directly the electrophoretic curve³.

Construction and principle. This adapter was constructed for the operation with the Coleman-Junior Spectrophotometer. The adapter consists of two parts: a densitometric and a registering one. Both parts can be separated or connected firmly together by means of the screw S. The Adapter is made of metal (dural, brass, steel).

During the operation, the densitometric part is plunged into the cuvette well of the spectrophotometer; it has the outside dimensions of the original cuvette adapter. Its function is very clearly apparent on the schematic diagram (Fig. 1). The coloured and transparent electrophoretic strip moves horizontally in front of the photocell by winding from the axis B onto the axis A (leading axis). On the upper end of the axis A, a wheel (diameter 34 mm) is fixed. By turning this wheel we are able to shift simultaneously both the electrophoretic and registering strips. Two opposite slits of 2.5 mm width and 32 mm length are cut out in the cylindrical mantle, which can be put on the lower end of this part of the Adapter.

The registering part is simply a flat box (dimensions: 70 × 50 × 14 mm) with the upper lid on hinges (Fig. 2). Through this lid a horizontal slit of 3 mm width and 60 mm length is cut out. On the upper margin of this slit, a millimeter scale is engraved so that the figures rise from 0 on the right to 60 on the left. At the left lower corner of this registering box, a simple device is arranged by means of which the inserted Adapter can be very firmly and reliably fastened to the galvanometer lever housing of the spectrophotometer (Fig. 2).

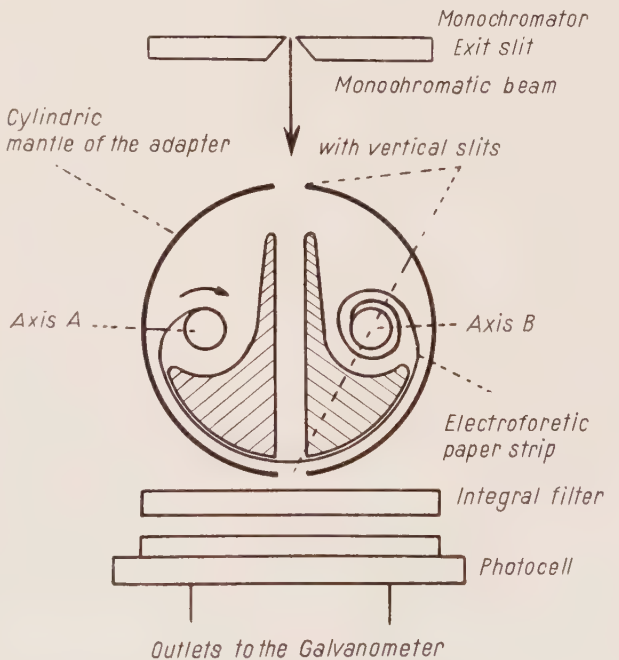


Fig. 1.—Schematic diagram of densitometric adapter.

The registering paper strip is 63 mm wide and 180 mm long with the left margin cut aslant in order to be insert-

¹ A. DITTMAR, Papier-Elektrophorese (Gustav Fischer-Verlag, Jena 1956). — F. WUHRMANN and CH. WUNDERLY, *Die Bluteiweisskörper des Menschen* (Benno Schwabe & Co., Basel 1952).

² M. D. SCHULZ *et al.*, Amer. J. clin. Path. 24, 1110 (1954).

³ CH. EGER, Exper. 12, 37 (1956). — G. CERIOTTI, Exper. 13, 44 (1957). — I became acquainted with this work when I had already overhanded my paper to the publishing board of Experientia.

ed into the fissure of the horizontal axis. The registering paper must be exactly as thick as the electrophoretic paper to assure the simultaneous shifting of both the strips. (I simply use the same paper, Whatman I.) Vertical ordinates 2.5 mm distant are preprinted on this paper.



Fig. 2.—The adapter inserted in the Spectrophotometer. Ready for the operation.

On the right side, below the horizontal (registering) slit, at a distance of 2 mm, there is a short parallel slit (2×5 mm), and the same kind of slit occurs near the upper end of the vertical (densitometric) slit. By means of these additional slits, we can test whether the shifting of both strips was really simultaneous during the measuring process.

Procedure.—A registering paper is put into the upper part of the Adapter and the coloured electrophoretic strip (35 mm wide, 180 mm long, with both ends cut, aslant) made transparent by a clarifier (paraffin oil, silicon oil, brom-naphthalene) into the densitometric part. The starting marks in both additional slits are made with a red pencil. Both parts are connected, the Adapter plunged into the cuvette well and fastened with the screw (Fig. 2).

The standard scale panel is changed for the special one which I have made by a photographic process. By means of it we can read directly the values of the protein concentrations and plot them in the millimeter scale from 0 to 60. (Up to the present time, I have made the evaluations at the wavelength of $620\text{ m}\mu$, I am now using $580\text{ m}\mu$. We dye the strips with Brom-Phenol-Blue.)

First we set the galvanometer index to 100% Transmittance (0 of the Optical Density) when the beam passes through the clear paper before the albumin. Then we shift both strips so that the shifting is always 2.5 mm which we check on the ordinates in the horizontal registering slit. We read the respective deviation of the galvanometer index, and plot them directly on the ordinate which lies close under the scale of the registering slit. In the normal electrophoresis, it is necessary to make about 40 or 50 such plottings. This takes about 10 min. With that the operation is finished. We take out the Adapter from the apparatus, make again the red marks in the testing slits, disconnect the parts and take out both the strips.

We join the registered points on the recording strip and thus obtain the resulting electrophoretic graph. We place the electrophoretic strip and the graph so gained above each other, and we can at once check whether we have worked correctly, provided, of course, that both the strips were shifted really simultaneously. In this case the starting and the ending red marks must fall on each other (Fig. 3).

Sometimes it happens that the boundary of singular protein fractions is not quite straight, on the contrary it may be accurate. If we were not able to repeat the electrophoresis, we can make the densitography with

Electrophoretogram recorded with Densitometric adapter *sec.* PALACKÝ on Coleman-Junior Spectrophotometer.

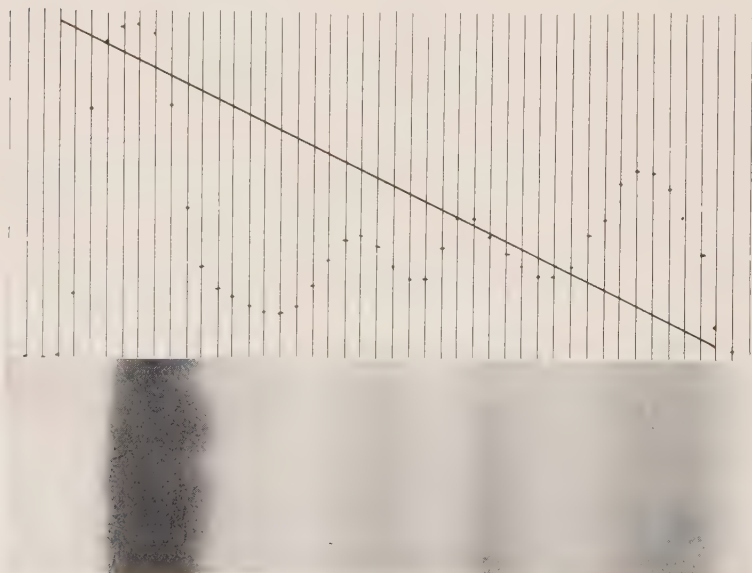


Fig. 3.— On purpose we didn't join the plotted points with a line. Note the starting and the end marks which lie precisely above each other proving that the shifting of both the strips was really simultaneous. The aslant (green) line on the registering paper helps us to get information about the proceeding of the operation. In the recording slit namely this oblique line runs from the left to the right. When, for instance, it is under the figure 30 we recognize that a half of the graph is over.

a slit of half-length (16 mm) by which we commit a lesser mistake than with the slit of full length. For this purpose, the 16 mm slits are cut in the cylindrical mantle rectangularly with the normal slits.

In successful electrophoretic strips with a perfect partition of protein bands, we can of course obtain still more exact values, when using a very narrow densitometric slit *e.g.* 1 mm. In this case it would be an advantage to concentrate the rays on the narrow slit in putting a cylindric lens (a glass round rod of 2.5 mm diameter) close in front of the strip.

The work with this densitometric Adapter Model IV is very quick and exact. It has the great advantage that we can directly obtain the electrophoretic graph. Even a non-qualified worker is able easily to learn the manipulation of it. The only disadvantage is that the device cannot be improvised but must be made very precisely. It is equal to the special and expensive densitometric apparatuses.

Acknowledgement. I am much obliged to render thanks to Mr. A. Šmíd for a very perfect technical construction of the models of my Adapter and for a very devoted and initiative technical collaboration by the merit of which my work, after numerous experiments, has been led to a successful end.

I am also much obliged to render thanks to Dr. J. BÁRTEK, Doctor-in-Chief of Central Laboratory of our Hospital for a friendly collaboration and precious advices.

A. PALACKÝ

Pediatric Department of the County Hospital at Uh. Hradiste, Czechoslovakia, December 28, 1956.

Zusammenfassung

Der Verfasser beschreibt eine Apparatur zur objektiven Auswertung der Papierelektrophoretogramme. Er benützt ein Coleman-Spektrophotometer und einen besonders entwickelten Adapter.

Informations - Informationen - Informazioni - Notes

STUDIORUM PROGRESSUS

Regulation of Blood Pressure and Hypertension

By C. HEYMANS*

Experiments performed in different laboratories¹ have shown that blood pressure is regulated reflexly by the action of arterial pressure itself on receptors sensitive to pressure located into the walls of the blood vessels of the aortic arch and carotid sinus areas.

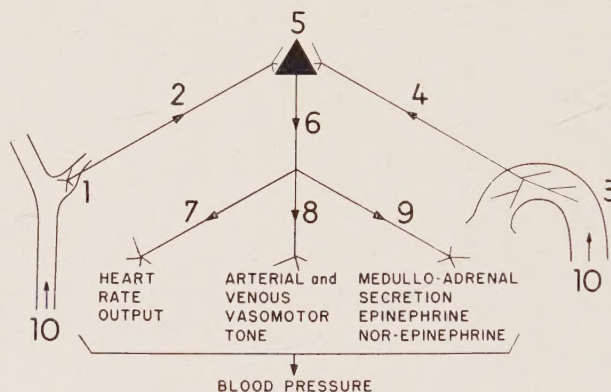
These pressor- or baro-receptors are connected by means of the aortic and carotid sinus nerves with the nerve centres regulating and maintaining arterial blood pressure at normal levels. Any deviation of arterial pressure induces, by means of the aortic and carotid sinus baroreceptors, compensatory reflexes and adjustments so as to restore arterial blood pressure at its normal levels. The efferent pathways of this self-adjustment of blood pressure, of this physiological blood pressure homeostasis, are the vagus and sympathetic nerves adjusting heart rate and cardiac output, the sympathetic vasomotor nerves adjusting the peripheral vasomotor tone of small arteries and veins, and thus the peripheral vascular resistance and the circulating blood volume, and the sympathetic nerves regulating the epinephrine and norepinephrine secretion of the suprarenal glands. These hormones also act, but by an humoral way, on heart rate, cardiac output and peripheral vascular resistance (Figure).

Further experimental observations showed that the aortic and carotid sinus baroreceptors and their nerves are not only the fundamental means of the blood pressure homeostasis, but also are the reflex buffer or moderator mechanisms of the systemic arterial pressure.

* Department of Pharmacology, Medical School University of Ghent (Belgium).

¹ H. E. HERING, *Die Karotissinusreflexe auf Herz und Gefäße* (Verlag Steinkopf, Dresden/Leipzig 1927). – E. KOCH, *Die Selbststeuerung des Kreislaufes* (Verlag Steinkopf, Dresden/Leipzig 1931). – C. HEYMANS, *Le Sinus carotidien* (Presses Universitaires, Paris 1929). – C. HEYMANS, J. J. BOUCKAERT, and P. REGNIERS, *Le Sinus Carotidien et la zone homologue cardio-aortique* (Doin et Cie, Paris 1933).

Paralysis of the baroreceptors or section of the aortic and carotid sinus nerves induces, indeed, a marked rise of blood pressure, thus a condition of acute or chronic arterial hypertension.



Schema of Self-regulation of Blood Pressure. 1 Carotid sinus baroreceptors; 2 Carotid sinus nerves; 3 Aortic baroreceptors; 4 Aortic-depressor nerves; 5 Cardio-vascular centres; 6 Efferent pathways of self-regulation of blood pressure; 7 Vago-sympathetic nerves to heart; 8 Vasomotor nerves to arteries and veins; 9 Sympathetic nerves to medullo-adrenal glands; 10 Arterial pressure acting on baroreceptors.

Everybody would agree that hypertension is a deviation of the arterial pressure, a 'resetting' of arterial pressure from normal to higher levels. In order to evaluate the origin and mechanism of this 'resetting' of blood pressure in hypertension, we ought to know in full details the physiological mechanisms maintaining blood pressure at normal levels. The physiology of blood pressure regulation is, thus, fundamental for the evaluation of the pathogenesis of hypertension.

As arterial pressure is maintained or restored reflexly at normal levels by the action of blood pressure itself on the aortic and carotid sinus baroreceptors, the question arises: how does arterial pressure act on these baroreceptors?

It has long been accepted that arterial pressure and its deviations act directly on the aortic and carotid sinus receptors. It has been stated, furthermore, that arterial pressure acts on the baroreceptors, by deforming and

thus stretching the arterial walls in which these receptors are located. Experimental evidence showed, indeed, that if deformation of the baroreceptive arterial walls by a rise of arterial pressure was prevented, the baroreceptors did not respond to the rise of pressure².

These experimental observations suggested that the responses of the baroreceptive arterial walls to pressure could play an important role in the mechanism of blood pressure homeostasis.

This suggestion has been investigated in our laboratory³. Experiments showed that an increase of tension of the baroreceptive arterial walls and, thus, an increase of pressure-response of the baroreceptive carotid sinus and aortic arterial walls induced by local application to the baroreceptive arterial walls of drugs, such as epinephrine, norepinephrine, serotonin or vasopressine, provokes a 'resetting' of the baroreceptive mechanisms of blood pressure regulation from normal to lower levels. Decrease of intramural tension and pressure-response of the baroreceptive arterial walls induced by local application of drugs such as papaverine, prisolone or nitrite, provokes, on the contrary, a 'resetting' of the baroreceptive mechanisms of blood pressure regulation from normal to higher levels.

Experiments performed on the isolated carotid sinus preparation also showed⁴ that epinephrine and norepinephrine increase the pressure-response of the arterial wall and decrease their distensibility to steady and pulsatile pressures.

Further experiments also disclosed that an increased intramural tension of the arterial wall, induced into the empty but baroreceptive normal innervated carotid sinus areas, also provokes a stimulation of the baroreceptors and thus a reflex fall of arterial pressure.

These experimental observations thus showed that the pressure-response of the baroreceptive arterial walls and the degree of their intramural tension or intramural stretch or distorsion plays a fundamental role in the physiological mechanisms of blood pressure homeostasis.

Under normal conditions the degree of intramural tension, stretch or distorsion of the baroreceptive arterial walls depends on the level of arterial pressure, and mainly on the pressure-response of the baroreceptive arterial walls themselves, thus on the resistance of the baroreceptive arterial walls to deformation by the intravascular pressure. Therefore, any alteration in pressure-response of the baroreceptive aortic and carotid sinus arterial walls will induce a 'resetting' of the blood pressure homeostasis to lower or to higher pressure levels. The biological condition of the baroreceptive arterial walls, thus, plays a fundamental role in the mechanisms of blood pressure regulation. The biological condition and, thus, the response of the baroreceptive arterial walls to pressure are, indeed, more important than the level of pressure itself for the physiological mechanisms of blood pressure homeostasis. A decrease of intramural

tension and resistance to distension of the arterial baroreceptive arterial walls, would induce a shift to a higher 'set' of baroreceptor function, a 'resetting' of the responses of the baroreceptors from the normal to a higher pressure level. Consequently, a higher arterial pressure would be necessary to provoke a response of the arterial pressure buffering mechanisms. This condition could be an important component in the mechanisms of arterial hypertension. Experiments of our laboratory support this suggestion, as they show that in chronic renal hypertension provoked in dogs, the arterial pressure is 'reset' from high to normal or low levels when the intramural tension and the pressure-response of the baroreceptive arterial walls is increased.

Recent experimental observations of McCUBBIN, GREEN, and PAGE⁶ are also in agreement with our hypothesis. They measured baroreceptor responses by means of electroneurograms in normotensive and chronically hypertensive dogs, and showed that the baroreceptor mechanisms are, indeed, reset to the hypertensive pressure level in animals with chronic renal hypertension and that the buffer baroreceptive reflexes tend to maintain, rather than to prevent, the chronic phase of renal hypertension and are, presumably, an important component in the mechanisms of chronic renal hypertension.

Let us hope that still more detailed information concerning the physiological mechanisms of blood pressure homeostasis, mainly concerning the factors maintaining or affecting the intramural tension and the pressure-response of the baroreceptive arterial walls, will also provide more definite information concerning the origin and mechanism of hypertension.

Résumé

L'auteur résume les mécanismes de l'homéostasie physiologique de la pression artérielle et indique que la réaction à la pression intravasculaire des parois artérielles barosensibles constitue un facteur prédominant de la régulation réflexe de la pression artérielle. Une élévation du seuil d'excitabilité des parois artérielles barosensibles pourrait constituer un élément causal important de l'hypertension artérielle.

⁵ G. MATTON, Arch. int. Pharmacodyn. 103, 13 (1955); 110, 472 (1957).

⁶ J. W. McCUBBIN, J. H. GREEN, and I. H. PAGE, Circul. Res. 4, 205 (1956).

Congressus

DEUTSCHLAND

9. Internationaler Kongress für Radiologie 1959

Der 9. Internationale Kongress für Radiologie findet in der Zeit vom 23. bis 30. Juli 1959 in München (Deutschland) unter dem Präsidium von Prof. B. RAJEWSKY, Frankfurt/Main (Deutschland) statt.

Generalsekretär ist Prof. Dr. HANS V. BRAUNBEHRENS, München. Alle Auskünfte über den Kongress erteilt das Kongresssekretariat in Frankfurt am Main, Forsthausstrasse 76.

Dr. VIKTOR LOECK

Corrigendum

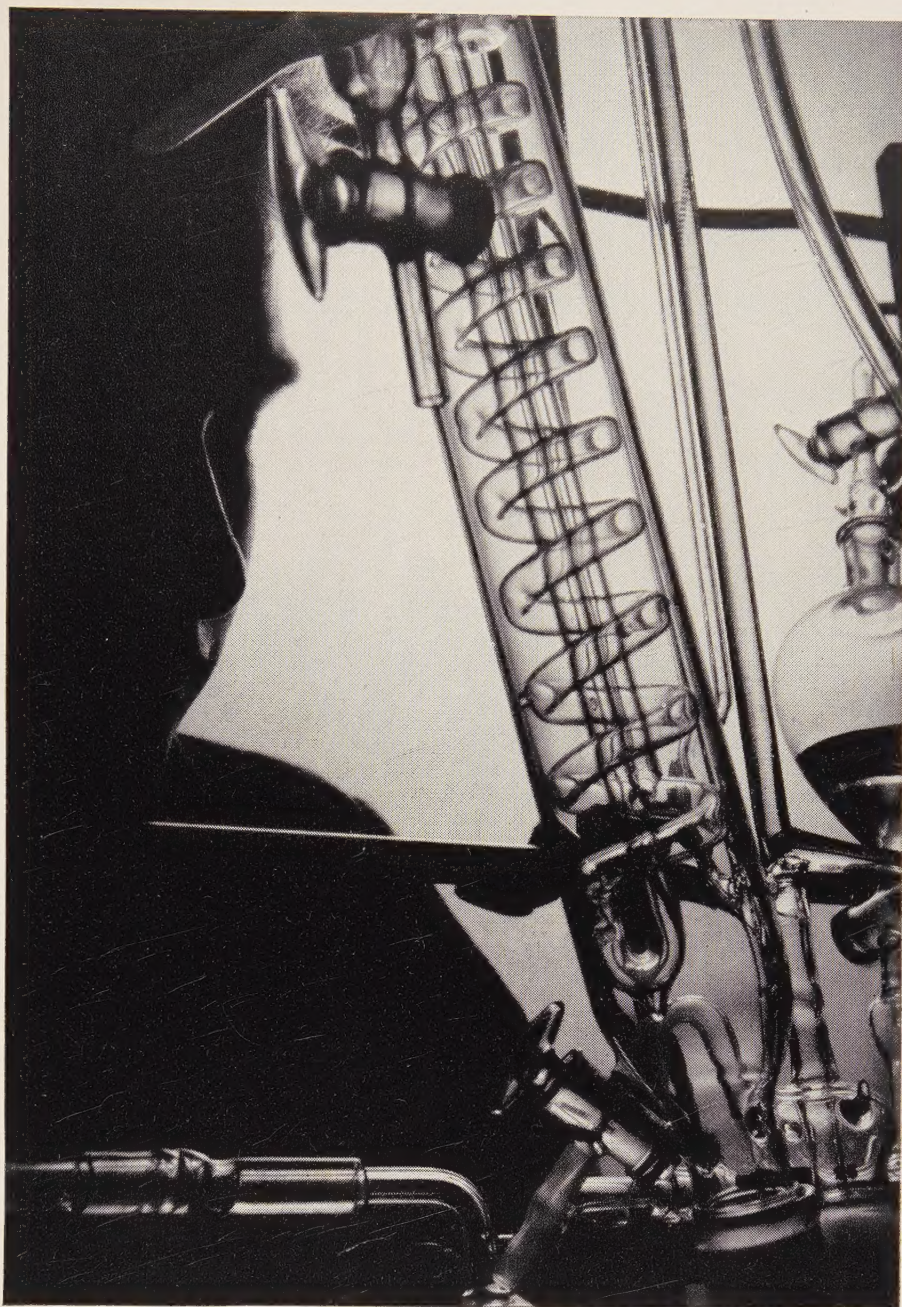
J. WILLEMS: *Orientierte Aufwachsungen auf hochpolymeren organischen Stoffen*, Experientia, Vol. XIII, Heft Nr. 7, S. 276 (1957).

Auf der rechten Spalte, unterste Zeile, sollte es heissen: $2c = 5,06 \text{ \AA}$ (anstatt $2c = 6,06 \text{ \AA}$).

² W. H. HAUSS, H. KREUZINGER, and H. ASTEROTH, Z. Kreislaufforsch. 38, 28 (1949).

³ C. HEYMANS and G. VAN DEN HEUVEL-HEYMANS, Arch. int. Pharmacodyn. 83, 520 (1950). – H. MAZELLA, S. C. WANG, C. HEYMANS, and G. R. DE VLEESCHOUWER, Arch. int. Pharmacodyn. 89, 122 (1952). – C. HEYMANS and G. VAN DEN HEUVEL-HEYMANS, Circulation 4, 581 (1951). – C. HEYMANS, A. L. DELAUNOIS, and G. VAN DEN HEUVEL-HEYMANS, Circul. Res. 1, 3 (1953). – C. HEYMANS and A. L. DELAUNOIS, Arch. int. Pharmacodyn. 96, 92 (1953); Science 114, 546 (1951). – C. HEYMANS, A. L. DELAUNOIS, and A. L. ROVATI, Arch. int. Pharmacodyn. 109, 245 (1957).

⁴ C. HEYMANS and A. L. DELAUNOIS, Arch. int. Pharmacodyn. 96, 92 (1953); Science 114, 546 (1951). – C. HEYMANS, A. L. DELAUNOIS, and A. L. ROVATI, Arch. int. Pharmacodyn. 109, 245 (1957).



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